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## Plant Characteristics

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## Key Findings

The key findings of this chapter are presented in the bullet points below.

- As the CO<sub>2</sub> content of the air rises, plant species may not experience photosynthetic acclimation—lower long-term rates of photosynthesis than occur when CO<sub>2</sub> concentrations are first increased—even under conditions of low soil nitrogen. In the event a plant cannot balance its carbohydrate sources and sinks, CO<sub>2</sub>-induced acclimation can shift limiting resources away from the site of photosynthesis to strengthen sink development or enhance other important plant processes.
- Atmospheric CO<sub>2</sub> enrichment may increase, decrease, or have no effect on leaf chlorophyll concentration. Even when leaf chlorophyll concentration is decreased, the reallocation of its nitrogen generally occurs without any adverse consequences, as most plants displaying this response typically continue to exhibit significant increases in photosynthesis and biomass production.
- Studies of several different tree species suggest enhanced carbohydrates stored in terminal branchlets or nitrogen stored in second-year leaves may be remobilized to facilitate greater first-flush needle or leaf growth the following year as a result of atmospheric CO<sub>2</sub> enrichment.
- At higher atmospheric CO<sub>2</sub> concentrations, plants tend to produce more and larger flowers, as well as other flower-related changes having significant implications for plant productivity and survival, almost all of which are positive.
- Higher concentrations of atmospheric CO<sub>2</sub> tend to reduce fluctuating asymmetry in plant leaves, leading to more symmetrical leaves that appear to be less susceptible to attack by herbivores.
- Atmospheric CO<sub>2</sub> enrichment enhances plant growth, development, and ultimate yield (in the case of agricultural crops) by increasing the concentrations of plant hormones that stimulate cell division, cell elongation, and protein synthesis.
- Atmospheric CO<sub>2</sub> enrichment increases the production of glomalin, a protein created by fungi living in symbiotic association with the roots of 80% of the planet's vascular plants, which is being released to almost every soil in the world in ever-greater quantities with the passage of time, where it is having a positive impact on the biosphere.
- Increasing atmospheric CO<sub>2</sub> concentrations likely will affect many leaf characteristics of agricultural plants, with the majority of the changes leading to higher rates and efficiencies of photosynthesis and growth, as well as numerous other changes in leaf characteristics that help promote increased resistances to herbivory and pathogen attack.
- Atmospheric CO<sub>2</sub> enrichment stimulates photosynthesis in nearly all plants, enabling them to produce more nonstructural carbohydrates that can be used to create important carbon-based secondary compounds, one of which is lignin.
- The highly positive impacts of atmospheric CO<sub>2</sub> enrichment on lipid concentrations and characteristics in various terrestrial and aquatic plants portend nothing but good for the managed and unmanaged components of Earth's biosphere as the air's CO<sub>2</sub> content continues to climb.
- The stresses of low temperature have been shown to be both alleviated and enhanced in plants growing under elevated CO<sub>2</sub> concentrations. It appears far more plants are benefiting from such stress alleviation than experience stress augmentation.
- The diverse results of several experimental studies do not paint a clear picture of what should be expected in the way of plant monoterpene emissions in a CO<sub>2</sub>-enriched and possibly warmer world of the future. Until a better understanding is obtained, it would be unwise to conclude much more about the situation.
- As the air's CO<sub>2</sub> content continues to rise, plant fitness, flower pollination, and nectar production should be enhanced, leading to increases in fruit, grain, and vegetable yields of agricultural crops, as well as similar increases in the productivity of the world's natural vegetation.
- Increases in the air's CO<sub>2</sub> content likely will stimulate nitrogen fixation in most herbaceous

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species that form symbiotic relationships with nitrogen-fixing soil bacteria—i.e., legumes—and this phenomenon likely will lead to increased nitrogen availability in soils and large increases in agricultural and natural ecosystem productivity. Likewise, increases in the air's CO<sub>2</sub> content will enhance the growth of Earth's leguminous trees, stimulating their fixation of nitrogen and their exudation of the resultant nitrogenous substances to the soils in which they grow, where they become available to neighboring non-leguminous vegetation, all of which promotes biospheric productivity.

- As the air's CO<sub>2</sub> content continues to rise, Earth's plants likely will reduce the amount of nitrogen invested in rubisco and other photosynthetic proteins while maintaining enhanced rates of photosynthesis and thereby increasing their photosynthetic nitrogen-use efficiencies.
- As the CO<sub>2</sub> content of the air increases, much of Earth's vegetation likely will display increases in biomass, and the larger plants likely will develop more extensive root systems and extract greater amounts of mineral nutrients from the soils in which they are rooted, enabling them to sustain their enhanced growth.
- Herbaceous plants often experience increases in foliar and fruit phenolic concentrations in response to atmospheric CO<sub>2</sub> enrichment, and these responses appear to have numerous positive implications for man and the biosphere. Similarly, future increases in the air's CO<sub>2</sub> concentration likely will enhance foliar phenolic concentrations in many trees and shrubs. This phenomenon should enhance woody-plant defense mechanisms that help deter herbivory, thereby improving forest health, robustness, and longevity.
- The wide range of results obtained by many past and current methods of detecting each year's start of spring has led to premature claims of spring's increasingly earlier annual occurrence over the course of the twentieth century. While these claims are used to support the associated claim of twentieth century CO<sub>2</sub>-induced global warming, real-world data fail to support the bulk of these contentions.
- With respect to managed agricultural crops and the wild plants of Earth's natural ecosystems, it appears the ongoing rise of the air's CO<sub>2</sub> concentration will have few negative impacts of any consequence on the nutritive value of their grains and foliage in terms of protein concentration. In tree crops such as citrus, CO<sub>2</sub>-induced changes in the activities of certain foliar proteins could lead to vast increases in yield potential.
- The growth and biomass production of herbaceous crops are generally enhanced by CO<sub>2</sub>-induced decreases in respiration during the dark (nighttime) period. However, scientists lack fundamental information on how respiration and the processes supported by it are physiologically controlled, thereby preventing sound interpretations of what seem to be species-specific responses of respiration to elevated CO<sub>2</sub>. Thus the precise role of plant respiration in augmenting the sink capacity of herbaceous plants remains uncertain.
- Both above and below the soil surface, coniferous trees appear to exhibit significant reductions in respiration in CO<sub>2</sub>-enriched air. However, deciduous trees exhibit increases and decreases, as well as cases of no change in respiration. Other studies of multiple tree species also suggest atmospheric CO<sub>2</sub> enrichment may either increase or decrease woody-plant respiration, but not to any great degree. The net result for Earth's trees generally likely would be of little significance.
- Elevated CO<sub>2</sub> nearly always increases root biomass, often even more than shoot biomass, by increasing the size of taproots and the number and size of lateral roots, along with fine-root biomass and a number of other important root properties.
- There is a reduced need for nitrogen investment in leaf rubisco in most plants growing in CO<sub>2</sub>-enriched air, because under such conditions plants typically reallocate some of their "surplus" nitrogen to other processes essential for optimal growth and development without compromising enhanced carbon gains via photosynthesis.
- Atmospheric CO<sub>2</sub> enrichment has been shown to increase the amount, size, weight, carbohydrate content, lipid content, protein content, earlier production, earlier germination, and greater percent germination of various plant seeds. All of these improvements bode well for the future of Earth's biosphere.

- As the air's CO<sub>2</sub> content rises, most of Earth's vegetation responds by exhibiting enhanced rates of photosynthesis and greater production of carbohydrates. Many of these carbohydrates are exported from leaves and needles to provide energy or carbon skeletons to facilitate increased biomass production, after which remaining carbohydrates generally are converted into starch and stored within leaves or roots for future use.
- In addition to sequentially reducing the openness of their stomata to restrict unnecessary water loss via excessive transpiration as the air's CO<sub>2</sub> content rises, some plants also reduce the density (number per area) of stomates on their leaves, but only to a certain degree, beyond which this latter phenomenon would be counterproductive.
- Atmospheric CO<sub>2</sub> enrichment typically increases plants' ability to produce greater amounts of various forms of sugar.
- Atmospheric CO<sub>2</sub> enrichment significantly enhances the condensed tannin concentrations of the vast majority of Earth's trees and grasses, providing them with stronger defenses against various herbivores both above and below ground. By reducing the amount of methane released to the atmosphere via ruminants browsing on tree leaves and grass, this phenomenon should reduce the rate of rise of the air's methane concentration and thereby decrease the impetus for methane-induced global warming.
- Thylakoid membranes of chloroplasts perform a host of important functions that influence the process of carbon fixation in plants, and in CO<sub>2</sub>-enriched air, they generally perform these functions more efficiently, enabling plants to overcome some of the deleterious effects of a host of plant stresses, including insufficient soil moisture, abnormally cold temperatures, abnormally hot temperatures, and ozone pollution.
- As the atmosphere's CO<sub>2</sub> concentration rises, most plants tend to exhibit increased rates of net photosynthesis and biomass production; on a per-unit-leaf-area basis, plants exposed to elevated CO<sub>2</sub> concentrations are likely to lose less water via transpiration because they tend to exhibit lower stomatal conductances. Therefore, the amount of carbon gained per unit of water lost per unit leaf area—water use efficiency—should increase significantly as the air's CO<sub>2</sub> content rises.
- Atmospheric CO<sub>2</sub> enrichment appears to enhance the between-cuttings savings of vegetative storage proteins (VSPs) in the roots of alfalfa crops, as well as the between-years storage of VSPs in the leaves of sour orange trees (and possibly other citrus species), so that when the next crop's or tree's growing season begins, the first flush of foliage gets a head start.
- Atmospheric CO<sub>2</sub> enrichment tends to increase wood density in both seedlings and mature trees, thereby also increasing a number of strength properties of their branches and trunks. However, different species of trees may respond somewhat differently to atmospheric CO<sub>2</sub> enrichment, and they can respond with still greater variety under different environmental conditions.

### Introduction

According to a draft of IPCC Working Group II's Summary for Policymakers (SPM) to accompany its 2014 report, "without adaptation, local temperature increases of 1°C or more above preindustrial levels are projected to negatively impact yields for the major crops (wheat, rice, maize) in tropical and temperate regions, although individual locations may benefit (*medium confidence*). With or without adaptation, climate change will reduce median yields by 0 to 2% per decade for the rest of the century, as compared to a baseline without climate change" (IPCC 2014, p. 10).

The research and analysis summarized in Chapter 1 of the current report challenged IPCC's predictions by showing how rising levels of atmospheric CO<sub>2</sub> help plants overcome abnormally hot temperatures and other plant stresses that might accompany a warming planet. It referenced tabular presentations in Appendices 3 and 4 reporting more than 5,000 individual plant biomass and photosynthetic responses reported in the peer-reviewed literature. That chapter went on to show how elevated atmospheric CO<sub>2</sub> and warmer temperatures benefit various important ecosystems including forests, grasslands, peatlands, wetlands, and soils.

The current chapter continues the analysis, presenting research on the effects of elevated CO<sub>2</sub> on a long list of plant characteristics ranging (in alphabetical order) from antioxidants and chlorophyll to water use efficiency and wood density. The sections

and subsections that follow report the results of hundreds of experiments involving plants grown in air enriched with carbon dioxide to levels expected to be experienced in the future as well as examination of yearly growth rings of long-lived trees revealing how they grew over prior years, decades, and even centuries as atmospheric CO<sub>2</sub> concentrations have risen. In the case of agricultural crops, grassland vegetation, and chaparral and desert species, the first of these techniques is typically employed, as it also is in the study of young tree seedlings. Growth-ring studies are reserved for more mature trees, some of which may have lived for several centuries.

The primary information being sought in these studies is rates of photosynthesis and biomass production and the efficiency with which the plants and trees utilize water. These studies also can provide information about substances produced in the growth process that affect how well it proceeds; substances deposited in the parts of agricultural crops that are harvested for human and animal consumption; and substances that determine whether the foliage or fruit of a certain crop or tree will be attractive to insect pests. Finally, these studies also can help determine whether forest soils will have sufficient nitrogen to sustain the long-term CO<sub>2</sub>-enhanced growth rates of long-lived trees.

This chapter powerfully rebuts claims that plant species typically experience photosynthetic acclimation—a decline in rates of photosynthesis over time to less than what would have been expected based on measurements made during short-term exposure to CO<sub>2</sub>-enriched air—even under conditions of low soil nitrogen. In the event a plant cannot balance its carbohydrate sources and sinks, CO<sub>2</sub>-induced acclimation represents a way of achieving that balance by shifting limiting resources away from the site of photosynthesis to strengthen sink development or enhance other important plant processes. This is especially important because many estimates of the costs and benefits of future global warming assume acclimation will limit the benefits of carbon dioxide fertilization or even turn carbon sinks into net emitters. The research that follows shows convincingly that this is not the case.

Similarly, this chapter addresses head-on the claim that water shortages in a warmer world will eventually stop the higher CO<sub>2</sub>-induced increase in plant growth and foreshadow declining plant productivity, with corresponding negative effects on food supply and human welfare. It seems Mother Nature anticipated the greater need for moisture that faster-growing plants would exhibit by minimizing

their loss of water via transpiration by lowering stomatal conductances. The amount of carbon gained per unit of water lost per unit of leaf area—water use efficiency—should increase significantly as the air's CO<sub>2</sub> concentration rises.

This chapter also looks closely at the impact of higher CO<sub>2</sub> levels on plants that are significant sources of nutrition for humans directly or indirectly as feed for cattle, sheep, and other domesticated animals. The evidence is overwhelmingly positive: Higher levels of atmospheric CO<sub>2</sub> already are enhancing crop yields of alfalfa, citrus, rice, soybeans, and wheat. Carbon dioxide fertilization promotes the production of more and larger flowers, more symmetrical leaves (which appears to reduce herbivory), more nonstructural carbohydrates, and positive impacts on lipid concentrations and characteristics, plant fitness, flower pollination, nectar production, and more. Rising levels of CO<sub>2</sub> in the air will have few negative impacts of any consequence on the nutritive value of grains and foliage in terms of protein concentration, and in some tree crops such as citrus, CO<sub>2</sub>-induced changes in the activities of certain foliar proteins could lead to vast increases in yield potential.

In short, IPCC's conclusions about the impact of future global warming on agriculture are far too pessimistic. Trends in the twentieth century and experimental research all point to a continued flourishing of crops important to human welfare as well as virtually all plants.

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IPCC. 2014. Summary for Policymakers. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability*. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Draft dated October 28, 2013.

## 2.1 Acclimation

Plants grown in elevated CO<sub>2</sub> environments often exhibit photosynthetic acclimation or down-regulation, typically characterized by somewhat lower long-term rates of photosynthesis than would have been expected based on measurements made during short-term exposure to CO<sub>2</sub>-enriched air. These downward adjustments result from modest long-term decreases in the activities and/or amounts of the

primary plant carboxylating enzyme rubisco.

Acclimation is said to have occurred when the photosynthetic rates of long-term CO<sub>2</sub>-enriched plants are found to be lower than those of long-term non-CO<sub>2</sub>-enriched plants when the normally CO<sub>2</sub>-enriched plants are measured during brief exposures to ambient CO<sub>2</sub> concentrations. The subsections below review research published on acclimation in agricultural, desert, grassland, and woody tree species.

### 2.1.1 Agricultural Species

- As the CO<sub>2</sub> content of the air rises, agricultural species may not experience photosynthetic acclimation, even under conditions of low soil nitrogen, but in the event a plant cannot balance its carbohydrate sources and sinks, CO<sub>2</sub>-induced acclimation represents a beneficial means of achieving that balance by shifting limiting resources away from the site of photosynthesis to strengthen sink development or enhance other important plant processes.

Several studies have examined the effects of elevated CO<sub>2</sub> on acclimation in agricultural crops. Ziska (1998), for example, reports soybeans grown at an atmospheric CO<sub>2</sub> concentration of 720 ppm initially exhibited photosynthetic rates 50% greater than those observed in control plants grown at 360 ppm. After the onset of photosynthetic acclimation, the CO<sub>2</sub>-enriched plants displayed subsequent photo-synthetic rates only 30% greater than their ambiently grown counterparts. Theobald *et al.* (1998) grew spring wheat at twice-ambient atmospheric CO<sub>2</sub> concentrations and determined elevated CO<sub>2</sub> reduced the amount of rubisco required to sustain enhanced rates of photosynthesis, which led to a significant increase in plant photosynthetic nitrogen-use efficiency. CO<sub>2</sub>-induced increases in photosynthetic nitrogen-use efficiency also have been reported in spring wheat by Osborne *et al.* (1998).

In a study incorporating both hydroponically and pot-grown wheat plants, Farage *et al.* (1998) demonstrated low nitrogen fertilization does not lead to photosynthetic acclimation in elevated CO<sub>2</sub> environments, as long as the nitrogen supply keeps pace with the relative growth rate of the plants. When spring wheat was grown at an atmospheric CO<sub>2</sub> concentration of 550 ppm in a free-air CO<sub>2</sub> enrichment (FACE) experiment with optimal soil nutrition and unlimited rooting volume, Garcia *et al.* (1998) found no evidence of photosynthetic

acclimation.

CO<sub>2</sub>-induced photosynthetic acclimation often results from insufficient plant sink strength, which can lead to carbohydrate accumulation in source leaves and the triggering of photosynthetic end-product feedback inhibition, which reduces net photosynthetic rates. Gesch *et al.* (1998) report rice plants, which have relatively limited potential for developing additional carbon sinks, grown at an atmospheric CO<sub>2</sub> concentration of 700 ppm exhibited increased leaf carbohydrate contents, which likely reduced *rbcS* mRNA levels and rubisco protein content. Similarly, Sims *et al.* (1998) report photosynthetic acclimation was induced in CO<sub>2</sub>-enriched soybean plants from the significant accumulation of nonstructural carbohydrates in their leaves. However, in growing several different *Brassica* species at 1,000 ppm CO<sub>2</sub>, Reekie *et al.* (1998) demonstrated CO<sub>2</sub>-induced acclimation was avoided in species having well-developed carbon sinks (broccoli and cauliflower) and appeared only in species lacking significant sink strength (rape and mustard).

Thus, photosynthetic acclimation does not appear to be a direct consequence of atmospheric CO<sub>2</sub> enrichment but rather an indirect effect of low sink strength, which results in leaf carbohydrate accumulation that can trigger acclimation.

In some cases, plants can effectively increase their sink strength, and thus reduce the magnitude of CO<sub>2</sub>-induced acclimation, by forming symbiotic relationships with certain species of soil fungi. Under such conditions, photosynthetic down-regulation is not triggered as rapidly, or as frequently, by end-product feedback inhibition, as excess carbohydrates are mobilized out of source leaves and sent belowground to symbiotic fungi. Louche-Tessandier *et al.* (1999) found photosynthetic acclimation in CO<sub>2</sub>-enriched potatoes was less apparent when plants were simultaneously colonized by a mycorrhizal fungus. Thus, CO<sub>2</sub>-induced acclimation appears to be closely related to the source:sink balance that exists within plants, being triggered when sink strength falls below, and source strength rises above, critical thresholds in a species-dependent manner.

Acclimation is generally regarded as a process that reduces the amount of rubisco and/or other photosynthetic proteins, which effectively increases the amount of nitrogen available for enhancing sink development or stimulating other nutrient-limited processes. Watling *et al.* (2000), for example, report a 50% CO<sub>2</sub>-induced reduction in the concentration of PEP-carboxylase, the primary carboxylating enzyme

in C<sub>4</sub> plants, within sorghum leaves. Maroco *et al.* (1999) documented CO<sub>2</sub>-induced decreases in both PEP-carboxylase and rubisco in leaves of the C<sub>4</sub> crop maize.

In some cases, acclimation to elevated CO<sub>2</sub> is manifested by an “up-regulation” of certain enzymes. When Gesch *et al.* (2002) took rice plants from ambient air and placed them in air enriched to 700 ppm CO<sub>2</sub>, for example, they noticed a significant increase in the activity of sucrose-phosphate synthase (SPS), a key enzyme involved in the production of sucrose. Similarly, Hussain *et al.* (1999) report rice plants grown at an atmospheric CO<sub>2</sub> concentration of 660 ppm displayed 20% more SPS activity during the growing season than did ambiently grown rice plants. Such increases in the activity of this enzyme could allow CO<sub>2</sub>-enriched plants to avoid the onset of photosynthetic acclimation by synthesizing and subsequently exporting sucrose from source leaves into sink tissues before they accumulate and trigger end product feedback inhibition.

Gesch *et al.* (2000) took ambiently growing rice plants and placed them in an atmospheric CO<sub>2</sub> concentration of 175 ppm, which reduced photosynthetic rates by 45%. After five days’ exposure to this sub-ambient CO<sub>2</sub> concentration, the plants manifested an up-regulation of rubisco, which stimulated photosynthetic rates by 35%. Thus, plant acclimation responses can involve both an increase or decrease in specific enzymes, depending on the atmospheric CO<sub>2</sub> concentration.

Many peer-reviewed studies suggest that as the CO<sub>2</sub> content of the air slowly but steadily rises, agricultural species may not exhibit photosynthetic acclimation, even under conditions of low soil nitrogen. If a plant can maintain a balance between its sources and sinks for carbohydrates at the whole-plant level, for example, acclimation should not be necessary; for if Earth’s atmospheric CO<sub>2</sub> content is rising by an average of only 1.5 ppm per year, most plants should be able to adjust their relative growth rates by the small amount that would be needed to prevent low nitrogen-induced acclimation from ever occurring, or expand their root systems by the small amount that would be needed to supply the extra nitrogen required to take full advantage of the CO<sub>2</sub>-induced increase in leaf carbohydrate production. And if a plant cannot initially balance its sources and sinks for carbohydrates at the whole-plant level, CO<sub>2</sub>-induced acclimation represents a beneficial secondary mechanism for achieving that balance through redistributing limiting resources away from the plant’s photosynthetic machinery to strengthen sink

development or enhance other important plant processes.

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### 2.1.2 Chaparral and Desert Species

- The few studies of acclimation that have been conducted on chaparral and desert plants indicate that although acclimation can sometimes be quite severe, other physiological changes, such as the reductions in stomatal conductance that typically produce large increases in water use efficiency, often more than compensate for the increases in photosynthesis caused by acclimation.

Roberts *et al.* (1998) conducted a FACE experiment in southern California, USA, exposing *Adenostoma fasciculatum* shrubs to atmospheric CO<sub>2</sub> concentrations of 360 and 550 ppm while they studied the nature of gas-exchange in this chaparral species. After six months of CO<sub>2</sub> fumigation, photosynthetic acclimation occurred. However, because of reductions in stomatal conductance and transpirational water loss, the CO<sub>2</sub>-enriched shrubs exhibited leaf water potentials less negative (and hence less stressful) than those of control plants. This CO<sub>2</sub>-induced water conservation phenomenon should enable this woody perennial to better withstand the periods of drought that commonly occur in this southern California region, and the photosynthetic down-regulation it exhibits should allow it to more equitably distribute the limiting resources it possesses among different essential plant physiological processes.

Huxman and Smith (2001) measured seasonal gas exchange during an unusually wet El Niño year in an annual grass (*Bromus madritensis* ssp. *rubens*) and a perennial forb (*Eriogonum inflatum*) growing within FACE plots established in the Mojave Desert, USA,

which they maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 550 ppm. The elevated CO<sub>2</sub> consistently increased net photosynthetic rates in the annual grass without inducing photosynthetic acclimation. Even as seasonal photosynthetic rates declined post-flowering, the reduction was much less in the CO<sub>2</sub>-enriched plants. Elevated CO<sub>2</sub> had no consistent effect on stomatal conductance in this species. In contrast, *Eriogonum* plants growing at 550 ppm CO<sub>2</sub> exhibited significant photosynthetic acclimation, especially late in the season, which led to similar rates of net photosynthesis in these plants in both CO<sub>2</sub> treatments. But in this species, elevated CO<sub>2</sub> reduced stomatal conductance over most of the growing season. And, therefore, although the two desert plants exhibited different stomatal and photosynthetic responses to elevated CO<sub>2</sub>, both experienced significant CO<sub>2</sub>-induced increases in water use efficiency and biomass production, highlighting the existence of different, but equally effective, species-specific mechanisms for responding positively to atmospheric CO<sub>2</sub> enrichment in a desert environment.

In another study conducted at the Mojave Desert FACE site, Hamerlynck *et al.* (2002) determined plants of the deciduous shrub *Lycium andersonii* grown in elevated CO<sub>2</sub> displayed photosynthetic acclimation, as maximum rubisco activity in the plants growing in the CO<sub>2</sub>-enriched air was 19% lower than in the plants growing in ambient air. The elevated CO<sub>2</sub> did not significantly impact rates of photosynthesis. Leaf stomatal conductance, by contrast, was consistently about 27% lower in the plants grown in the CO<sub>2</sub>-enriched air. During the last month of the spring growing season, the plants in the elevated CO<sub>2</sub> plots displayed leaf water potentials less negative than those exhibited by the control plants growing in ambient air. Hence, as the CO<sub>2</sub> content of the air increases, *Lycium andersonii* likely will respond by exhibiting significantly enhanced water use efficiency, which should greatly increase its ability to cope with the highly variable precipitation and temperature regimes of the Mojave Desert. The acclimation observed within the shrub's photosynthetic apparatus should allow it to reallocate more resources to sustaining greater amounts of biomass. Thus it is likely future increases in the air's CO<sub>2</sub> content will favor a “greening” of the American Mojave Desert.

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### 2.1.3 Grassland Species

- As the air's CO<sub>2</sub> content rises, grassland species may not exhibit photosynthetic acclimation if they can maintain a balance between their carbohydrate sources and sinks. But if this balancing act is not possible, acclimation represents a beneficial way of achieving that balance by redistributing limiting resources away from a plant's photosynthetic machinery to strengthen its sink development and/or its nutrient-gathering activities.

In nearly every reported case of photosynthetic acclimation in CO<sub>2</sub>-enriched grassland species, rates of photosynthesis displayed by plants grown and measured at elevated concentrations of CO<sub>2</sub> are typically greater than those exhibited by control plants grown and measured at ambient CO<sub>2</sub> concentrations (Davey *et al.*, 1999; Bryant *et al.*, 1998). But as mentioned in prior sections, CO<sub>2</sub>-induced photosynthetic acclimation often results from insufficient plant sink strength that can lead to carbohydrate accumulation in source leaves and the triggering of photosynthetic end-product feedback inhibition, which reduces rubisco activity and rates of net photosynthesis (Roumet *et al.*, 2000).

As one example of this phenomenon, Rogers *et al.* (1998) report perennial ryegrass grown at an atmospheric CO<sub>2</sub> concentration of 600 ppm and low soil nitrogen content exhibited leaf carbohydrate contents and rubisco activities 100% greater and 25% less, respectively, than those observed in control plants grown at 360 ppm CO<sub>2</sub> prior to a cutting event. But following the cutting, which effectively reduced the source:sink ratio of the plants, leaf carbohydrate contents in CO<sub>2</sub>-enriched plants decreased and rubisco activities increased, completely ameliorating the photosynthetic acclimation in this species.

However, at high soil nitrogen, photo-synthetic acclimation to elevated CO<sub>2</sub> did not occur. Thus, photosynthetic acclimation appears to result from the inability of plants to develop adequate sinks at low soil nitrogen, and is not necessarily induced directly by atmospheric CO<sub>2</sub> enrichment.

In some cases, plants can effectively increase their sink strength and thus reduce the magnitude of CO<sub>2</sub>-induced acclimation by forming symbiotic relationships with certain species of soil fungi. Under such conditions, photosynthetic down-regulation is not triggered as rapidly, or as frequently, by end-product feedback inhibition, as excess carbohydrates are mobilized out of source leaves and sent below-ground to the symbiotic fungi. Staddon *et al.* (1999), for example, report photosynthetic acclimation was not induced in CO<sub>2</sub>-enriched *Plantago lanceolata* plants inoculated with a mycorrhizal fungus, but it was induced in control plants that were not inoculated with the fungus. Thus, CO<sub>2</sub>-induced acclimation appears to be closely related to the source:sink balance that exists within plants, and which is triggered when sink strength falls below, and source strength rises above, certain critical thresholds in a species-dependent manner.

As the air's CO<sub>2</sub> content slowly but steadily rises, therefore, these peer-reviewed studies suggest grassland species may not exhibit photosynthetic acclimation if they can maintain a balance between their sources and sinks for carbohydrates at the whole-plant level. But in the event this balancing act is not possible, acclimation represents a beneficial secondary means of achieving that balance by redistributing limiting resources away from a plant's photosynthetic machinery to strengthen its sink development and/or its nutrient-gathering activities.

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### 2.1.4 Tree Species

Aspen/poplar trees, growing out-of-doors and rooted in the ground, generally show little long-term photosynthetic acclimation (gradual reduction in initial experimental CO<sub>2</sub>-induced growth stimulation), and sometimes none at all. Although there can be a steep multiyear decline in the initial huge CO<sub>2</sub>-induced growth stimulation of citrus trees, the subsequent long-term equilibrium growth enhancement can be quite substantial. And in oak trees, both long-term and total-lifetime CO<sub>2</sub> enhancement of the air surrounding various species have provided evidence for a total lack of photosynthetic acclimation, indicating the many positive and enduring benefits of atmospheric CO<sub>2</sub> enrichment. Also, even though there may be a partial acclimation of the photosynthetic process in pine trees in some CO<sub>2</sub>-enrichment experiments, the down-regulation is typically neither complete nor wholly detrimental, for it transfers nitrogen from the sites of photosynthesis to the sinks for photosynthates, where it is most needed when soil infertility is limiting primary productivity. Finally, studies of spruce trees reveal atmospheric CO<sub>2</sub> enrichment increases their photo-synthetic rates, even in the case of partial photo-synthetic acclimation, which enables them to transport greater amounts of soluble carbohydrates, including glucose, to active sink tissues to support their enhanced growth and development even on nutrient-poor soils.

#### 2.1.4.1 Aspen/Poplar

- Aspen/poplar trees, growing out-of-doors and rooted in the ground, generally show little long-term acclimation (reduction in CO<sub>2</sub>-induced growth stimulation), and sometimes none at all.

Trees grown for long periods of time in elevated CO<sub>2</sub> environments sometimes exhibit some degree of photosynthetic acclimation or down-regulation, typically characterized by modestly reduced rates of photosynthesis compared to what might be expected on the basis of short-term exposure to CO<sub>2</sub>-enriched air. These reductions result from a long-term decrease in the activity and/or amount of the primary plant carboxylating enzyme *rubisco*. This section explores this phenomenon as it applies to various species of aspen/poplar trees.

Takeuchi *et al.* (2001) grew quaking aspen (*Populus tremuloides*) trees for two years in 30-meter-diameter free-air CO<sub>2</sub> enrichment (FACE) plots near Rhinelander, Wisconsin (USA), maintained at atmospheric CO<sub>2</sub> concentrations of either 360 or 560 ppm. They found elevated CO<sub>2</sub> stimulated the growth and closure of the trees' canopies, as indicated by light intensities measured near the seedlings' lowermost branches only 17% and 9% of values observed near their uppermost branches in the ambient and CO<sub>2</sub>-enriched environments, respectively, which led to the light availability for photosynthesis decreasing with canopy depth, but more so for the CO<sub>2</sub>-enriched seedlings. Nevertheless, seasonal photosynthetic rates were always greater in the CO<sub>2</sub>-enriched seedlings, although the growth stimulation was much greater in the upper canopy than in the lower (26 and 3%, respectively). Photosynthetic acclimation also occurred in the CO<sub>2</sub>-enriched seedlings in a depth-dependent manner, with less acclimation occurring in the upper as opposed to the lower canopy, as indicated by decreases in foliar *rubisco* content of 28 and 50%, respectively, in those two locations. Yet in spite of this significant acclimation, the elevated CO<sub>2</sub> still led to a greater total net carbon uptake, and the CO<sub>2</sub>-enriched seedlings grew 18% taller than the seedlings exposed to ambient air.

At the EuroFACE facility near Viterbo in Central Italy, Wittig *et al.* (2005) grew stands of closely spaced (1 m x 1 m) individuals of three *Populus* species—white poplar (*P. alba*), black poplar (*P. nigra*), and robusta poplar (*P. x euramericana*)—from the time of planting through canopy closure to coppice (aboveground tree harvest) at atmospheric CO<sub>2</sub> concentrations of 370 and 550 ppm for three full years. Based on measurements of leaf area index and various photosynthetic parameters made at regular intervals, photosynthetic rates of different leaf classes were determined for monthly intervals and summed to obtain annual canopy photosynthesis or gross primary production (GPP) in each of the three years of the

study.

The 10 researchers note, “significant stimulation of GPP driven by elevated CO<sub>2</sub> occurred in all 3 years, and was greatest in the first year (223-251%), but markedly lower in the second (19-24%) and third years (5-19%).” The decline in CO<sub>2</sub>-induced growth stimulation was *not* due to photosynthetic acclimation; it was simply a consequence of canopy closure and the increased shading of leaves that accompanied it.

Averaged across all species and plots, the CO<sub>2</sub>-induced stimulation of annual GPP was 234%, 22%, and 11% in 1999, 2000, and 2001, respectively. Averaged over the full three-year period, the GPP enhancements for *P. alba*, *P. nigra*, and *P. x euramericana* were, respectively, 17%, 17%, and 25%. The scientists note these results “were consistent with independent measurements of net primary production, determined independently from biomass increments and turnover.” Thus, Wittig *et al.* say their results suggest, “with selection, nutrient and moisture supply, coppice managed plantation poplars have the potential for large and sustained increases in GPP” in response to atmospheric CO<sub>2</sub> enrichment.

Over the following year, as the trees sent up new sprouts and renewed their growth after coppicing, Davey *et al.* (2006) measured total daily photosynthetic carbon assimilation together with several other related physiological parameters and processes. They found “diurnal photosynthesis in poplar trees grown in elevated CO<sub>2</sub> over four growing seasons showed a sustained increase in photosynthesis of between 35 and 60% prior to coppicing,” and “this increase in daily photosynthesis is maintained during the re-growth following coppicing in *P. x euramericana*.”

These observations and their other data indicate, as the seven scientists describe it, “no long-term photosynthetic acclimation to CO<sub>2</sub> occurred in these plants,” and “poplar trees are able to ‘escape’ from long-term, acclamatory down-regulation of photosynthesis through a high capacity for starch synthesis and carbon export.” They also note, “Wittig *et al.* (2005) showed that the canopy photosynthetic carbon gain in these species is proportional to wood increment, implying that the increased photosynthesis will result in more carbon in wood.” Therefore, the seven scientists say their findings “show that the acclamatory loss of the initial increase in photosynthetic rate under elevated CO<sub>2</sub> is not inevitable,” and “poplar species, selected for rapid growth, may be well suited to a future elevated CO<sub>2</sub> environment and particularly suited to afforestation

projects aimed to increase carbon uptake into wood in the near term.”

Throughout the same year and over one additional year, Liberloo *et al.* (2005) studied the regrowth of the trees under unfertilized and fertilized conditions. During those first and second growing seasons after coppicing, they found the elevated CO<sub>2</sub> treatment significantly increased the trees’ leaf area index, with relative differences between the CO<sub>2</sub>-enriched and control trees ranging from +1.7 to +38.7%, +4.7 to +38.5%, and +3.9 to +45% for *P. alba*, *P. nigra*, and *P. x euramericana*, respectively, for unfertilized and fertilized conditions, respectively. In addition, they report the increased leaf area index “supported increased aboveground biomass production,” but only in the fertilized treatment. In light of these findings, they remark that if the CO<sub>2</sub>-induced growth enhancement after canopy closure continues to hold true in subsequent years, it “will have important implications for the carbon balance of terrestrial ecosystems, because forests could behave as a larger carbon sink under future atmospheric conditions.”

Liberloo *et al.* (2006) report results for the second three-year growth rotation of the trees. They found fertilization did not affect the growth of the second-rotation trees, “likely because of the high rates of fertilization during the previous agricultural land use.” In contrast, they write, “elevated CO<sub>2</sub> enhanced biomass production by up to 29%, and this stimulation did not differ between above- and below-ground parts.” They also report the net rate of carbon assimilation was “on average for all species stimulated up to 30% during the third year of the second rotation,” and “after six years of fumigation, measurements of photosynthetic parameters along the canopy profile could not detect any clear sign of acclimation to elevated CO<sub>2</sub>” for any of the three species. Thus, they conclude, “poplar trees are able to optimally profit from future high CO<sub>2</sub> concentrations, provided that they are intensively managed, planted in regions with high incident radiation and supplied with sufficient nutrients and water.” Such “high-density poplar coppice cultures,” in their opinion, “offer possibilities to mitigate the rise of atmospheric CO<sub>2</sub> by producing renewable bio-energy in an economically feasible way, whereby the elevated CO<sub>2</sub> stimulation might sustain over several rotation cycles.”

Working at the same site but studying only robusta poplar that had been growing there for five years, Calfapietra *et al.* (2005) measured the trees’ photosynthetic responses to an approximate 200-ppm

increase in the air's CO<sub>2</sub> concentration in mid-July of the study's fifth year, comparing their results with what was observed at the beginning of the experiment, both with and without supplemental nitrogen fertilization. As they describe their findings, "even after such a long period of exposure, leaves of *Populus x euramericana* have not shown clear signs of photosynthetic acclimation." They also report CO<sub>2</sub> enrichment "significantly decreased stomatal conductance both on upper and lower canopy leaves," which together with the CO<sub>2</sub>-induced stimulation of photosynthesis implies a significant sustained increase in leaf water use efficiency throughout the trees' canopies. They say their results "suggest that the photosynthetic acclimation of poplar plantations is unlikely to occur in an atmosphere enriched in CO<sub>2</sub> and thereby will not influence the response of poplar plantations to increasing atmospheric CO<sub>2</sub> concentrations either over the long term or under conditions of nitrogen deposition."

At the Aspen FACE site near Rhinelander, Wisconsin (USA), Kets *et al.* (2010) measured diurnal changes in light-saturated net photosynthesis (P<sub>n</sub>) rate under both ambient and elevated atmospheric CO<sub>2</sub> and/or ozone (O<sub>3</sub>) concentrations over wide ranges of stomatal conductance, water potential, intercellular CO<sub>2</sub>, leaf temperature, and vapor pressure difference between leaf and air in two clones (271 and 42E) of quaking aspen (*Populus tremuloides* Michx.) trees that differed in their sensitivity to ozone and had been growing under the aforementioned experimental conditions for seven to eight years. This work revealed P<sub>n</sub> was typically enhanced by 33 to 46% in the CO<sub>2</sub>-enriched treatment over the course of the study, and there was a small increase in leaf chlorophyll concentration as well.

Noting "previous Aspen FACE studies have reported 25–36% increases in P<sub>n</sub> (Noormets *et al.*, 2001; Takeuchi *et al.*, 2001; Sharma *et al.*, 2003; Ellsworth *et al.*, 2004)," the six scientists emphasize the aerial fertilization effect of atmospheric CO<sub>2</sub> enrichment on P<sub>n</sub> observed in their study "has rather been increasing in time than decreasing," stating this phenomenon may be caused by the "slight but significant increase in leaf chlorophyll content per leaf area, which is rather positive acclimation in photosynthetic apparatus than negative acclimation." They cite the studies of Centritto and Jarvis (1999) and Eichelmann *et al.* (2004) in support of this conclusion.

Darbah *et al.* (2010) analyzed photosynthesis data they and others had collected at the Aspen FACE site over a period of 11 years for the same two quaking

aspen clones, which were exposed to all combinations of ambient and elevated (560 ppm) CO<sub>2</sub> and ambient and elevated (1.5 times ambient) ozone (O<sub>3</sub>). In addition, they studied leaf stomatal conductance under the same conditions. This work revealed, as they describe it, "no long-term photosynthetic and stomatal acclimation to elevated CO<sub>2</sub>, O<sub>3</sub> or CO<sub>2</sub> + O<sub>3</sub> in aspen trees exposed to elevated CO<sub>2</sub> and/or O<sub>3</sub> gases for 11 years," and the aspen trees "have sustained their maximum instantaneous photosynthesis stimulation for over a decade." In discussing their findings, Darbah *et al.* say they support the observations of Liberloo *et al.* (2007), who measured a 49% increase in net photosynthetic rate in poplar trees after six years of exposure to elevated CO<sub>2</sub>; Sholtis *et al.* (2004), who reported a 44% stimulation of net photosynthesis in sweetgum trees after three years of exposure to elevated CO<sub>2</sub>; Crous and Ellsworth (2004), who found a photosynthetic enhancement of 51 to 69% in *Pinus taeda* trees after six years of exposure to elevated CO<sub>2</sub>; and Davey *et al.* (2006) and Paoletti *et al.* (2007), of whose work Darbah *et al.* state, "there was no photosynthetic acclimation (down-regulation) occurring in *Quercus ilex* under long-term CO<sub>2</sub> enrichment." In addition, they remark, even in white clover (*Trifolium repens*), Ainsworth *et al.* (2003) found photosynthetic stimulation "remained after nine years of exposure to elevated CO<sub>2</sub>."

Thus, as more long-term experiments are conducted on long-lived woody plants growing out-of-doors and rooted in the ground, where their roots are not artificially confined to a limited volume of soil, it is becoming abundantly clear they generally do not experience a complete cessation of the initial photosynthetic stimulation provided them by the extra CO<sub>2</sub> to which they are exposed in CO<sub>2</sub> enrichment studies. They often show very little long-term reduction in CO<sub>2</sub>-induced growth stimulation, and sometimes no reduction at all, as is evidenced in these several studies of aspen/poplar trees.

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#### 2.1.4.2 Citrus

- Although there can be a steep multiyear decline in the initial CO<sub>2</sub>-induced growth stimulation of citrus trees, the subsequent long-term equilibrium growth enhancement can be substantial.

Trees grown for long periods of time in air enriched with CO<sub>2</sub> sometimes exhibit photosynthetic acclimation or down-regulation. This phenomenon is characterized by modestly reduced rates of photosynthesis (compared to what is observed during short-term exposure to CO<sub>2</sub>-enriched air), which result from a slow decline in the activity and/or amount of the primary plant carboxylating enzyme *rubisco*. This

section reports what has been learned about this phenomenon in the few experiments that have studied it in citrus trees.

Jifon *et al.* (2002) grew seedlings of sour orange (*Citrus aurantium* L.) and sweet orange (*Citrus sinensis* L.) for nearly three months in glasshouses maintained at atmospheric CO<sub>2</sub> concentrations of either 360 or 700 ppm, where they were either inoculated with arbuscular mycorrhizal fungi or left non-inoculated as control plants. Thus they studied the effects of both elevated CO<sub>2</sub> and fungal presence on photosynthesis and growth in these two citrus species of contrasting fungal acceptance: sour orange, which displays strong associations with mycorrhizal symbionts, and sweet orange, which exhibits relatively weaker relationships with such fungi.

This protocol revealed elevated CO<sub>2</sub> increased photosynthetic rates in non-mycorrhizal and mycorrhizal sour orange tree seedlings by 18% and 118%, respectively. Elevated CO<sub>2</sub> enhanced photosynthetic rates in non-mycorrhizal and mycorrhizal sweet orange seedlings by 50% and 67%, respectively.

In terms of biomass production, the mycorrhizal sour orange seedlings exposed to ambient CO<sub>2</sub> displayed 18% less growth than the non-mycorrhizal control seedlings, but at elevated CO<sub>2</sub>, the mycorrhizal seedlings displayed 15% more growth than the non-mycorrhizal seedlings. Thus atmospheric CO<sub>2</sub> enrichment more than compensated for the carbon costs associated with maintaining the mycorrhizal fungal symbiosis in the sour orange seedlings, and sweet orange seedlings exposed to elevated CO<sub>2</sub> exhibited the same increase in biomass with or without fungal inoculation, indicating this species is less dependent upon fungal symbiosis in eliciting CO<sub>2</sub>-induced growth responses.

As the air's CO<sub>2</sub> content continues to rise, therefore, both of these citrus species likely will respond by exhibiting enhanced rates of photosynthesis and biomass production. In sour orange trees, photosynthetic and growth responses to elevated CO<sub>2</sub> will likely be greater when seedlings are involved in symbiotic relationships with soil fungi. Jifon *et al.* found the degree of CO<sub>2</sub>-induced photosynthetic acclimation or down-regulation in sour orange tree seedlings was significantly reduced when mycorrhizal fungi were present, as they served as a carbon sink for excess carbohydrates produced by photosynthesis, thereby alleviating the notorious end-product inhibition of photosynthesis. Thus, it is likely increasing atmospheric CO<sub>2</sub> concentrations may increase growth in nearly all tree species throughout

their normal life spans, for most are involved in symbiotic relationships with one or more types of mycorrhizal fungi.

Adam *et al.* (2004) measured numerous plant physiological processes and properties throughout the fourteenth year of a long-term study of the effects of a 75% increase (from 400 ppm to 700 ppm) in the air's CO<sub>2</sub> concentration on the growth and development of sour orange trees that had been grown from the seedling stage to maturity under well-watered and fertilized conditions out-of-doors at Phoenix, Arizona (USA) in clear-plastic-wall open-top enclosures. They then compared their results with those of similar measurements made in earlier years of the study.

In the second year of the experiment, net photosynthesis rates were 2.84 times greater in the CO<sub>2</sub>-enriched enclosures than in the ambient-air enclosures. By the sixth year of the study, however, this enhancement ratio had declined to 1.75, and in the fourteenth year it had dropped to 1.45. Plotting similarly declining above-ground woody biomass ratios against these net photosynthesis ratios, Adam *et al.* derived a linear relationship with an  $r^2$  value of 0.997 that yielded a CO<sub>2</sub>-induced woody biomass enhancement ratio of 1.78 at the 14-year point of the study. This value for the woody biomass ratio previously had been found by Idso and Kimball (2001) to have been essentially constant from year 10 to year 14, leading Adam *et al.* to conclude the CO<sub>2</sub>-induced net photosynthesis ratio they derived (1.45) likely also had been essentially constant over this period, indicating the final equilibrium level at which it had apparently stabilized.

Other evidence for the down-regulation of photosynthesis in the CO<sub>2</sub>-enriched sour orange tree leaves was provided by the observation that in year 14 of the study, the pooled mean of the concentration of the large subunit of rubisco in the CO<sub>2</sub>-enriched leaves was only 78% of that observed in the ambient-air leaves, and the concentration of the small subunit of rubisco was reduced by 34% in the CO<sub>2</sub>-enriched leaves compared to the ambient-air leaves. In addition, the full and initial activities of rubisco under CO<sub>2</sub> enrichment were reduced, as were leaf chlorophyll *a* and total nitrogen concentrations.

Adam *et al.* conclude, "long-term CO<sub>2</sub> enrichment can result in photosynthetic down-regulation in leaves of trees, even under non-limiting nitrogen conditions." At the final equilibrium level of acclimation experienced in the sour orange trees of this long-term study, the 75% enhancement of the air's CO<sub>2</sub> concentration still produced an equivalent percentage increase (or possibly slightly more) in

both wood and fruit production (78 and 80%, respectively).

Three years later, Kimball *et al.* (2007) described the final state of the Phoenix sour orange trees at the termination of that 17-year-long CO<sub>2</sub> enrichment experiment. In terms of total biomass production, which was the primary focus of their summary report, they state the CO<sub>2</sub>-enriched to ambient-treatment ratio of annual wood plus fruit production peaked in years 2–4 of the experiment at a value of approximately 2.4, and following that peak “there was a decline through year 8.” Thereafter, however, they found the annually produced-biomass ratios were, as they describe it, “more or less at a plateau that corresponded with the value of the ratio at final harvest of 1.69.”

In terms of harvestable yield, the four researchers write, “the cumulative amount of biomass due to fruit production over the duration of the experiment was increased 85% due to elevated CO<sub>2</sub>,” and this increase “was entirely from an increase in fruit number.” In addition, they report, “the vitamin C content of the fruit was increased 7% based on samples taken from the fourth through the 12th years of the experiment,” citing the study of Idso *et al.* (2002). Not only were many more oranges produced by the trees in the CO<sub>2</sub>-enriched chambers, but many more better-quality oranges were produced.

In the concluding discussion of one of the major implications of the study, Kimball *et al.* write, “rather than a continual acclimation”—i.e., rather than a gradual long-term decline in the aerial fertilization effect of the extra 300 ppm of CO<sub>2</sub> supplied to the CO<sub>2</sub>-enriched trees—“there was a sustained enhancement of about 70% in annual fruit and incremental wood production over the last several years of the experiment.” This observation led them to conclude, “the effects of elevated CO<sub>2</sub> on trees can be large and sustained for many years,” as they demonstrated to be the case with the sour orange trees they studied: Over the entire last decade of the experiment, there was a 70% sustained increase in total biomass production and an 85% increase in fruit production in response to the 75% increase in the air’s CO<sub>2</sub> content employed throughout the study.

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### 2.1.4.3 Oak

- Both long-term and total-lifetime CO<sub>2</sub> enhancement of the air surrounding various species of oak trees have provided evidence for a lack of photosynthetic acclimation, indicative of the many positive and enduring benefits of atmospheric CO<sub>2</sub> enrichment.

Working in Italy with mature downy oak (*Quercus pubescens*) trees located at two distances from natural CO<sub>2</sub>-emitting springs, Stylinski *et al.* (2000) measured various physiological and biochemical properties of trees that had been exposed to atmospheric CO<sub>2</sub> concentrations of approximately 370 and 700 ppm for their entire 40- to 50-year life spans. The oak trees exposed to the elevated CO<sub>2</sub> concentration exhibited net photosynthetic rates 36 to 77% greater than those exhibited by the trees growing at the ambient CO<sub>2</sub> concentration. The CO<sub>2</sub>-enriched trees showed no signs of photosynthetic down-regulation. There were no significant differences between the CO<sub>2</sub>-enriched and ambient trees with respect to their leaves’ rubisco activity, rubisco content, total nitrogen content, chlorophyll content, and carotenoid content.

Polle *et al.* (2001) collected acorns from mature holly oak (*Quercus ilex*) trees that had been growing under the same set of conditions in Italy, exposed to ambient and twice-ambient atmospheric CO<sub>2</sub> concentrations for their entire lifetimes. After germinating those acorns, the seedlings they produced were grown for eight months at both atmospheric CO<sub>2</sub> concentrations to see if atmospheric CO<sub>2</sub> enrichment of the parent trees had any effect on their offspring’s

response to atmospheric CO<sub>2</sub> enrichment.

The researchers found elevated CO<sub>2</sub> increased whole-plant biomass by 158 and 246% in seedlings derived from acorns produced in ambient and twice-ambient atmospheric CO<sub>2</sub> concentrations, respectively, and the final biomass of the CO<sub>2</sub>-enriched seedlings derived from the acorns produced in the CO<sub>2</sub>-enriched air was 25% greater than that of the CO<sub>2</sub>-enriched seedlings derived from the acorns produced in ambient air. In addition, their gas exchange measurements indicated the CO<sub>2</sub>-enriched seedlings derived from the acorns produced on the CO<sub>2</sub>-enriched trees exhibited less-pronounced photosynthetic acclimation to elevated CO<sub>2</sub> than did the CO<sub>2</sub>-enriched seedlings derived from the acorns produced on the trees exposed to ambient air.

Blaschke *et al.* (2001) studied the effects of long-term atmospheric CO<sub>2</sub> enrichment on gas exchange in both species of mature oak trees—*Quercus ilex* (a strongly drought-tolerant evergreen species) and *Quercus pubescens* (a less drought-tolerant deciduous species)—both of which were growing near CO<sub>2</sub>-emitting springs in Italy. They made physiological and biochemical measurements on trees that had been exposed to atmospheric CO<sub>2</sub> concentrations of approximately 370 and 700 ppm for their 30- to 50-year lifetimes. The CO<sub>2</sub>-enriched *Q. pubescens* and *Q. ilex* trees exhibited net photosynthetic rates 69% and 26% greater, respectively, than those displayed by trees exposed to ambient CO<sub>2</sub>, in spite of CO<sub>2</sub>-induced decreases of 30% and 15% in their respective foliar rubisco concentrations. In addition, stomatal conductances of CO<sub>2</sub>-enriched *Q. pubescens* trees were approximately 23% lower than those of ambient trees, and stomatal conductances of *Q. ilex* trees displayed no CO<sub>2</sub>-sensitivity. Both species exhibited increased water use efficiencies in the elevated CO<sub>2</sub> environment closest to the CO<sub>2</sub>-emitting springs. Blaschke *et al.*'s results clearly demonstrate fully mature trees continue to exhibit enhanced rates of photosynthesis and increases in water use efficiency even after decades of exposure to elevated atmospheric CO<sub>2</sub> concentrations.

Marek *et al.* (2001) employed open-top chambers to boost the air's CO<sub>2</sub> content approximately twofold for five years around 30-year-old *Quercus ilex* trees growing in perennial evergreen stands. They found the extra CO<sub>2</sub> increased rates of net photosynthesis in sun-exposed and shaded leaves by 68% and 59%, respectively. After measuring short-term photosynthetic rates at various atmospheric CO<sub>2</sub> concentrations, they found no evidence of photosynthetic acclimation in the leaves of the mature

trees. In addition, they determined the trees' light compensation point—the light level at which photosynthetic carbon uptake is matched by respiratory carbon loss—was 24% and 30% lower in sun-exposed and shaded leaves, respectively, of CO<sub>2</sub>-enriched trees than it was in corresponding leaves of the trees growing in ambient air.

As the atmosphere's CO<sub>2</sub> concentration continues to increase, therefore, the work of Marek *et al.* suggests its stimulatory effect on oak tree photosynthesis will persist over the long term. In addition, because elevated CO<sub>2</sub> significantly lowered the light compensation point in mature oak trees, which would allow them to exhibit net carbon gains earlier in the morning and maintain them later into the evening, the stimulatory effect of elevated CO<sub>2</sub> on daily carbon uptake will be further enhanced. Together, these observations suggest carbon sequestration by oak trees, and perhaps other tree species, may be more substantial in future CO<sub>2</sub>-enriched air than has been projected.

After burning a Florida (USA) scrub-oak ecosystem (dominated by *Quercus myrtifolia*, *Q. chapmanii*, and *Q. geminata*) to the ground, Ainsworth *et al.* (2002) erected open-top chambers on the site and fumigated them with ambient (380 ppm) and CO<sub>2</sub>-enriched (700 ppm) air to study the effects of elevated CO<sub>2</sub> on community regeneration. During the third and fourth years of the study, they found elevated CO<sub>2</sub> consistently increased photosynthetic rates in *Q. myrtifolia* and *Q. chapmanii* by as much as 150% without inducing any degree of photosynthetic acclimation, although acclimation was observed in *Q. geminata*. After three years of exposure to elevated CO<sub>2</sub>, the three oak species taken together exhibited an average increase in their rates of photosynthesis of 53%. Thus, at higher CO<sub>2</sub> concentrations, regenerating scrub-oak communities likely will exhibit enhanced rates of photosynthesis that will persist throughout canopy closure and maturity. These increases in photosynthesis likely will enhance community biomass production. The five researchers conclude the sustained increases in photosynthesis exhibited by *Q. myrtifolia* and *Q. chapmanii* have "translated to increased growth in these species, and there is no suggestion that this trend is changing." Carbon sequestration in regenerating and maturing scrub-oak ecosystems is likely to continue to increase with future increases in the air's CO<sub>2</sub> concentration.

Paoletti *et al.* (2007) measured rates of net photosynthesis in upper sunlit leaves of mature *Quercus ilex* trees growing close to (5 m) and further away from (130 m) a CO<sub>2</sub>-emitting spring, where the

trees had experienced lifetime exposure to atmospheric CO<sub>2</sub> concentrations of approximately 1,500 and 400 ppm, respectively. This was done during a two-week period in June 2002 at the end of the spring rains, when midday air temperatures rose above 40°C. The net photosynthetic rates of the leaves on the trees growing closest to the CO<sub>2</sub> spring were approximately 250% greater than those of the leaves on the trees growing 125 meters further away, where the air's CO<sub>2</sub> concentration was 1,100 ppm less than it was in the vicinity of the trees nearest the spring. The four Italian researchers conclude, "the considerable photosynthetic stimulation at the very high CO<sub>2</sub> site suggests no photosynthetic down-regulation over long-term CO<sub>2</sub> enrichment."

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- 2.1.4.4 Pine**
- Even though there may be a partial acclimation of the photosynthetic process in pine trees in some CO<sub>2</sub>-enrichment experiments, the down-regulation is typically neither complete nor wholly detrimental, for it transfers nitrogen from the sites of photosynthesis to the sinks for photosynthates, where it is most needed when soil infertility is limiting primary productivity.
- Studies of the effects of atmospheric CO<sub>2</sub> enrichment on the growth and development of pine trees over the past several years have focused chiefly on three species: *Pinus radiata* (Monterey pine), *Pinus sylvestris* (Scots pine), and *Pinus taeda* (Loblolly pine).
- Turnbull *et al.* (1998) grew seedlings of *P. radiata* in open-top chambers near Bromley, Christchurch (New Zealand) for four years at atmospheric CO<sub>2</sub> concentrations of 360 and 650 ppm. Near the conclusion of this period they measured photosynthetic parameters in current-year and one-year-old needles at each of the CO<sub>2</sub> concentrations to determine the effects of elevated CO<sub>2</sub> and leaf age on photosynthesis. This work revealed CO<sub>2</sub>-induced increases in needle photosynthetic rate of 63% and 31% in current and one-year-old needles, respectively, suggesting needle age might be an important determinant of photosynthetic acclimation in this species. As further evidence for this hypothesis, they found atmospheric CO<sub>2</sub> enrichment did not induce changes in rubisco content or activity in current-year needles, but it did reduce rubisco content and activity by about 40% in one-year-old needles. After four years of CO<sub>2</sub> enrichment, the CO<sub>2</sub>-induced photosynthetic enhancement persisted in each year's new flush of needles, while it experienced a partial down-regulation in older needles.
- Reporting further on the same experiment, Griffin *et al.* (2000) noted a decline in older-needle rubisco content in the elevated CO<sub>2</sub> treatment, as well as a 40% reduction in needle stomatal conductance. The first of these changes implies that with the redistribution of nitrogen away from rubisco (which allows it to perform other vital functions), it is likely *P. radiata* will maintain high rates of net carbon uptake while using less nitrogen and other resources in doing so. Coupling these observations with the reduction in water loss due to CO<sub>2</sub>-induced decreases in stomatal conductance, *P. radiata* seedlings probably will grow more efficiently with less water inputs in future atmospheres containing greater con-

centrations of CO<sub>2</sub>.

At the conclusion of the four-year study of *P. radiata* in Christchurch, New Zealand, Greenep *et al.* (2003) took cuttings from the four-year-old Monterey pines that had experienced lifetime exposure to either ambient or elevated atmospheric CO<sub>2</sub> concentrations and grew them for one year in their respective CO<sub>2</sub> treatments in open-top chambers, where they were irrigated daily and fertilized every three months. They found the “photosynthetic rate in young needles during summer, autumn and spring was 34, 43 and 38% higher, respectively, in trees grown at elevated CO<sub>2</sub> than in trees grown at ambient CO<sub>2</sub>,” and “in older needles, the corresponding photosynthetic rate increases were 26, 47 and 49%.” In addition, water use efficiency was 49% higher in the foliage of the elevated CO<sub>2</sub> treatment, although there was no change in needle stomatal conductance.

These responses were comparable to those observed in the parent trees when they were approximately the same size and age as the second-generation trees. However, there were signs of photosynthetic acclimation in the older needles of the parent trees when they were larger (in their third and fourth years) but physiologically younger than the second-generation trees, which by the time of this study (having been derived from four-year-old trees and grown for an additional year) were in their fifth year of life. Thus, the researchers suggested the down-regulation of photosynthesis observed in the parent trees in their third and fourth years “was a result of a shift in the proportion of young to old needles as the trees increased in size.”

In concluding, they hypothesize, “in small trees, close proximity of active sinks, such as developing buds, to a proportionally small reservoir of source tissue (mature foliage) would increase the overall sink strength and reduce the extent of photosynthetic acclimation (down-regulation) at elevated CO<sub>2</sub>.” They note, for example, “within an immature *P. radiata* canopy, young needles may represent as much as 4 to 10 times the biomass of older needles,” but “as the canopy matures this ratio approaches equality (Turnbull *et al.*, 1998).” Hence, they conclude, “down-regulation of photosynthesis at elevated CO<sub>2</sub> is related to tree size rather than tree age or duration of exposure,” and “the capacity for enhanced photosynthesis in trees growing in elevated CO<sub>2</sub> is unlikely to be lost in subsequent generations.”

Jach and Ceulemans (2000) studied three-year-old *Pinus sylvestris*, Scots pine, seedlings rooted in the ground and grown in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350

and 750 ppm for two additional years to determine the long-term effects of elevated CO<sub>2</sub> on photosynthesis in this important European timber species. To make the experimental results more representative of the natural world, no nutrients or irrigation water were applied to the soils during their investigation.

During the second year of atmospheric CO<sub>2</sub> enrichment, the photosynthetic rates of the current-year and one-year-old CO<sub>2</sub>-enriched needles were 62% and 65% greater, respectively, than the rates displayed by the needles on the seedlings growing in ambient air. When photosynthesis was measured at atmospheric CO<sub>2</sub> concentrations reciprocal to growth CO<sub>2</sub> concentrations, the researchers detected photosynthetic acclimation in the CO<sub>2</sub>-enriched seedlings, as evidenced by a 21% reduction in their photosynthetic rates. However, Jach and Ceulemans note, “the stimulatory effect of elevated CO<sub>2</sub> on photosynthesis substantially exceeded the magnitude of down-regulation.” Even with photosynthetic acclimation, the rate of net photosynthesis in the CO<sub>2</sub>-enriched seedlings was more than 40% greater than the rate measured in the control seedlings exposed to ambient air.

In a parallel study of Scots pine, Gielen *et al.* (2000) worked with six-year-old seedlings rooted in the ground within open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 750 ppm for three years to determine the long-term effects of elevated CO<sub>2</sub> on chlorophyll fluorescence and needle characteristics. No nutrients or irrigation water were applied to the soil. At the end of the period, a detailed seasonal analysis indicated elevated CO<sub>2</sub> did not significantly impact the photochemical quantum efficiency of photosystem II, nor did it affect any parameters associated with chlorophyll fluorescence. This indicates atmospheric CO<sub>2</sub> enrichment did not modify the light-dependent reactions of photosynthesis in this species.

With respect to needle characteristics, on the other hand, elevated CO<sub>2</sub> reduced needle nitrogen and chlorophyll contents by 33% and 26%, respectively. These observations suggest the light-independent reactions of photosynthesis were being modified by long-term exposure to elevated CO<sub>2</sub> in a manner indicating photosynthetic acclimation. But this acclimation of the photosynthetic process allows for the redistribution of limiting resources—such as nitrogen—away from what thus becomes a more efficient photosynthetic apparatus, so this important nutrient can be utilized in other areas of the tree where it is needed more. Thus, this CO<sub>2</sub>-induced phenomenon can have a positive effect on growth

because it allows trees to produce more biomass under conditions of low soil fertility than would otherwise be possible under ambient CO<sub>2</sub> concentrations, due to the mobilization of nitrogen out of photosynthetically active leaves and into actively expanding sink tissues.

Maier *et al.* (2002) constructed open-top chambers around 13-year-old *Pinus taeda*, loblolly pines, growing on infertile sandy soil and fumigated them for two more years with air containing either 350 ppm or 550 ppm of CO<sub>2</sub>, while half of the trees at each CO<sub>2</sub> concentration received supplemental soil fertilization. They found the elevated CO<sub>2</sub> increased branch needle area by 13%, and soil fertilization increased it by 38%. Applied together, the two treatments enhanced branch needle area by 56%. In addition, the extra CO<sub>2</sub> enhanced the trees' net photosynthesis rates by 82%, with the trees showing no signs of photosynthetic acclimation over the two-year duration of the study.

Crous and Ellsworth (2004) studied the photosynthetic rates of different-age needles measured at different crown positions on the 19-year-old (in 2002) loblolly pine trees at the Duke Forest FACE facility in the sixth year of that long-term study, comparing them with the results of similar measurements made over the prior five years. Although there was some evidence of photosynthetic down-regulation in one-year-old needles across the fifth to sixth year of CO<sub>2</sub> exposure, the two researchers report "strong photosynthetic enhancement in response to elevated CO<sub>2</sub> (e.g., +60% across age classes and canopy locations) was observed across the years."

Looking at the concept of acclimation from a somewhat different perspective, Lichter *et al.* (2008) noted progressive nitrogen limitation (PNL) may "accompany carbon sequestration in plants and soils stimulated by CO<sub>2</sub> fertilization, gradually attenuating the CO<sub>2</sub> response," after which they described what they learned about the PNL hypothesis from a nine-year CO<sub>2</sub> enrichment study.

The nine researchers report their data pertaining to forest-floor carbon pools indicate the existence of "a long-term steady-state sink" of about 30 g C per m<sup>2</sup> per year, which represents, they write, "a substantial increase in forest-floor C storage under elevated CO<sub>2</sub> (i.e. 29%)," and which they attribute to "increased litterfall and root turnover during the first 9 years of the study." Of the mineral soil C formed over this period, they note "approximately 20% has been allocated to stable pools that will likely remain protected from microbial activity and associated release as CO<sub>2</sub>."

The research team also found "a significant widening of the C:N ratio of soil organic matter in the upper mineral soil under both elevated and ambient CO<sub>2</sub>." This suggests, as they describe it, "enhanced rates of soil organic matter decomposition are increasing mineralization and uptake to provide the extra N required to support the observed increase in primary productivity under elevated CO<sub>2</sub>." At the Duke Forest FACE site, for example, Pritchard *et al.* (2008) say this CO<sub>2</sub>-induced increase in productivity amounts to approximately 30% annually, and they add there is "little evidence to indicate a diminished response through time," citing Finzi *et al.* (2007), who found the same to be true at the long-term forest FACE studies being conducted at Rhinelander, Wisconsin (USA), Oak Ridge National Laboratory (USA), and Tuscania (Italy).

It thus appears several of Earth's forests thought to have access to less-than-adequate soil nitrogen supplies may be able to acquire the extra nitrogen they need to maintain the sizable increases in their growth rates that are driven by elevated concentrations of atmospheric CO<sub>2</sub>. In the case of North Carolina's Duke Forest, for example, "even after nine years of experimental CO<sub>2</sub> fertilization," as Lichter *et al.* describe it, "attenuation of the CO<sub>2</sub>-induced productivity enhancement has not been observed," as also has been noted by Finzi *et al.* (2006). This finding at this location is extremely significant, because the growth of pine-hardwood forests in the southeastern United States often removes so much nitrogen from the soils in which they grow that they induce what Finzi and Schlesinger (2003) have described as "a state of acute nutrient deficiency that can only be reversed with fertilization," which was not employed in the Duke Forest FACE study.

Even though there may sometimes be a partial acclimation of the photosynthetic process in pine trees in some CO<sub>2</sub>-enrichment experiments, the research described above suggests the down-regulation seems never to be complete. The phenomenon may even play a positive role in shifting much-needed nitrogen from the sites of photosynthesis to the sinks for photosynthates in situations where soil fertility is a limiting factor to primary productivity.

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#### 2.1.4.5 Spruce

- Studies of spruce trees reveal atmospheric CO<sub>2</sub> enrichment increases their photosynthetic rates, even in the case of partial photosynthetic acclimation, which enables them to transport greater amounts of soluble carbohydrates, including glucose, to active sink tissues to support their enhanced growth and development even on nutrient-poor soils.

The large increases in net photosynthesis exhibited by trees growing in CO<sub>2</sub>-enriched air have been claimed to disappear gradually over extended periods of time, in a process known as acclimation or down-regulation. The following section reviews the findings of several studies of spruce trees that bear on this question.

Spunda *et al.* (1998) monitored 15-year-old Norway spruce trees in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm for four additional years, to study the long-term effects of elevated CO<sub>2</sub> on photosynthetic acclimation. When they measured the trees' photosynthetic rates at reciprocal growth CO<sub>2</sub> concentrations, they found the CO<sub>2</sub>-enriched shoots displayed a reduction of 18% compared to shoots grown in ambient air, indicating the presence of CO<sub>2</sub>-induced photosynthetic acclimation. Likewise, when analyzing photosynthetic pigments, they found the total amounts of chlorophylls and carotenoids in needles produced in CO<sub>2</sub>-enriched air to be 17% and 14% less, respectively, than the amounts found in needles produced in ambient air. Nevertheless, when measured at growth CO<sub>2</sub> concentrations, current-year CO<sub>2</sub>-enriched shoots still displayed rates of net photosynthesis 78% greater than those exhibited by shoots of trees grown in ambient air.

Egli *et al.* (1998) rooted several saplings of different Norway spruce genotypes directly into calcareous or acidic soils in open-top chambers and exposed them to atmospheric CO<sub>2</sub> concentrations of 370 or 570 ppm and low or high soil nitrogen contents, in order to determine the effects of elevated CO<sub>2</sub> and soil quality on the saplings' photosynthesis and growth rates. They found the elevated CO<sub>2</sub> stimulated light-saturated rates of net photosynthesis

under all conditions, with all genotypes exhibiting stimulations as great as 35%. Elevated CO<sub>2</sub> also led to a down-regulation of photosynthesis. Rates of leaf photosynthesis still remained higher for the trees grown in the CO<sub>2</sub>-enriched air, despite the occurrence of this phenomenon. This increase in photosynthesis ultimately contributed to greater instantaneous water-use efficiencies of the trees grown in elevated CO<sub>2</sub>, which were also promoted by CO<sub>2</sub>-induced decreases in needle stomatal conductance. All these processes operating together consistently led to increased aboveground biomass production, regardless of genotype, soil type, and nitrogen content.

Wiemken and Ineichen (2000) grew Norway spruce seedlings for three years in growth chambers maintained at atmospheric CO<sub>2</sub> concentrations of 280, 420, and 560 ppm. In addition, the seedlings received either low, medium, or high levels of nitrogen fertilization. This work revealed nitrogen fertilization did not affect the concentrations of any sugars within mature needles of the seedlings, but atmospheric CO<sub>2</sub> enrichment significantly enhanced needle glucose contents in a season-dependent manner.

In the highly productive growing phases characteristic of spring and early summer, for example, glucose contents in mature needles of the CO<sub>2</sub>-enriched trees were not significantly different from those observed in needles of the trees exposed to atmospheric CO<sub>2</sub> concentrations of 280 and 420 ppm. In late summer, fall, and winter, however, glucose concentrations in needles on the CO<sub>2</sub>-enriched trees were 40% to 50% higher than those of needles on trees subjected to ambient and sub-ambient CO<sub>2</sub> concentrations (420 and 280 ppm, respectively). These seasonal fluctuations in needle glucose concentrations suggested to the two researchers glucose levels may be mediating a seasonal photosynthetic down-regulation in spruce needles, as had been noted previously by others.

It would appear that as the air's CO<sub>2</sub> content continues to rise, Norway spruce trees likely will increase their photosynthetic rates, which should result in greater needle concentrations of soluble carbohydrates, including glucose. During favorable growing conditions associated with spring and early summer, the additional glucose produced by trees growing in CO<sub>2</sub>-enriched air likely will be mobilized and sent to active sinks to support their growth and development. As growing conditions become less favorable in late summer, glucose may not be mobilized from needles as rapidly as it was during the spring and early summer, which may lead to a seasonal photosynthetic down-regulation in this species.

In spite of this intimation of a temporary seasonal down-regulation of photosynthesis in Norway spruce seedlings, it seems logical to expect they will still exhibit greater biomass production at higher atmospheric CO<sub>2</sub> concentrations, as demonstrated by the findings of Spanda *et al.* (1998) and Egli *et al.* (1998) and the work of Tjoelker *et al.* (1998). The latter scientists studied *Picea mariana* (black spruce) together with quaking aspen, paper birch, tamarack, and jack pine in controlled-environment chambers for three months at atmospheric CO<sub>2</sub> concentrations of 370 ppm or 580 ppm and day/night temperatures ranging from 18/12°C to 30/24°C. They found the 57% increase in the CO<sub>2</sub> content of the air significantly stimulated net photosynthesis in all of the studied species by an average of 28%, regardless of temperature, over the three-month study period. Elevated CO<sub>2</sub> decreased leaf nitrogen levels in all species, causing differing degrees of photosynthetic down-regulation. However, the mobilization of nitrogen from the trees' leaves or needles, coupled with the sustained enhancement of photosynthetic rates, led to increased photosynthetic nitrogen-use efficiencies in all plants grown in elevated CO<sub>2</sub>. In addition, the elevated CO<sub>2</sub> decreased stomatal conductance by 10% to 25% in all species, leading to 40% to 80% increases in their instantaneous water-use efficiencies. As in essentially all such studies conducted to date, these benefits of atmospheric CO<sub>2</sub> enrichment more than compensated for the incomplete acclimation that sometimes occurs.

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### 2.1.5 Source/Sink Relations

- Plants with large sink capacity typically respond strongly to atmospheric CO<sub>2</sub> enrichment by increasing their photosynthetic rates; but when sink capacity is low, they reduce their rates of photosynthesis via photosynthetic acclimation, yielding up more of the nitrogen and other nutrients they possess to help build sink structures such as roots, tubers, or aboveground fruit.

Plants grown in elevated CO<sub>2</sub> for extended durations often, but not always, exhibit some degree of photosynthetic acclimation or down-regulation, typically characterized by reduced rates of photosynthesis resulting from decreased activity and/or amount of the primary plant carboxylating enzyme rubisco. When this phenomenon occurs, leaf nitrogen content often decreases, as nitrogen previously invested in rubisco is transferred to other parts of the plant. Photosynthetic acclimation to elevated CO<sub>2</sub> can be induced by insufficient plant sink strength, which often leads to carbohydrate accumulation in source leaves and reductions in net rates of photosynthesis. Acclimation also can result from the physical constraints of growing plants in pots or by limiting their access to important nutrients such as nitrogen. This section reviews the results of various studies of source:sink relationships on plant growth responses to atmospheric CO<sub>2</sub> enrichment.

In a simple experiment designed to determine how sink size naturally influences plant growth response to elevated CO<sub>2</sub>, Reekie *et al.* (1998) grew various *Brassica* species of differing sink capacity in growth chambers receiving atmospheric CO<sub>2</sub> concentrations of 350 and 1,000 ppm. After four weeks, elevated CO<sub>2</sub> had increased the total dry weights of all *Brassica* species. However, at final harvests made after six or 12 weeks of CO<sub>2</sub> fumigation, significant CO<sub>2</sub>-induced increases in total dry weight persisted only in species possessing well-developed carbon sinks. Thus, the authors' data suggest species with inherently low sink strength are more likely to experience CO<sub>2</sub>-induced acclimation than are species with inherently large sink strength that can naturally utilize the additional carbohydrates produced under CO<sub>2</sub>-enriched conditions to progressively increase their biomass.

In a non-invasive manipulation of plant source:sink ratios, Gesch *et al.* (1998) grew rice plants for one month in growth chambers having atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm before switching half of the plants in each chamber to

the other CO<sub>2</sub> growth concentration. Within 24 hours of switching, plants moved into the elevated CO<sub>2</sub> environment displayed a mean 15% increase in photosynthetic rate and a mean 19% reduction in *rbcS* mRNA, whereas previously CO<sub>2</sub>-enriched plants switched to ambient CO<sub>2</sub> exhibited photosynthetic rates 10% lower than those of plants grown continuously at ambient CO<sub>2</sub> and an analogous 19% increase in the amount of *rbcS* transcript. These observations suggest rice plants grown continuously in elevated CO<sub>2</sub> experienced sink limitations to growth.

In the FACE experiment of Rogers *et al.* (1998), perennial ryegrass growing at atmospheric CO<sub>2</sub> concentrations of 360 and 600 ppm was frequently cut to determine how physical changes in source:sink relationships influence transient growth responses to elevated CO<sub>2</sub>. Regardless of cutting events, plants grown in elevated CO<sub>2</sub> with high soil nitrogen availability exhibited no signs of CO<sub>2</sub>-induced photosynthetic acclimation. In contrast, when measured one day prior to cutting, plants grown in elevated CO<sub>2</sub> with low soil nitrogen availability exhibited 25% reductions in leaf rubisco content. One day after cutting, this acclimation response was completely eliminated, probably in response to rapid carbohydrate utilization to repair cut leaves. Similarly, Bryant *et al.* (1998) report simulated grazing events reversed CO<sub>2</sub>-induced decreases in rubisco activity in leaves of chalk grassland species growing on nutrient-poor soils. The results of these studies suggest photosynthetic acclimation likely results from an indirect effect of low soil nitrogen on sink development, rather than from a direct effect of elevated CO<sub>2</sub> on leaf photosynthetic capacity.

Farage *et al.* (1998) investigated the role of nitrogen supply in inducing photosynthetic acclimation in CO<sub>2</sub>-enriched wheat. In one experiment, plants were grown in pots placed within growth chambers receiving 350 ppm or 650 ppm CO<sub>2</sub> and were irrigated with fixed amounts of low- or high-nitrogen solutions on a regular basis, which is standard protocol for experiments utilizing potted plants. In the other experiment, plants were grown hydroponically at 350 ppm or 650 ppm CO<sub>2</sub> to eliminate any root restriction effects on growth. The plants were placed in nutrient solutions containing low or high concentrations of nitrogen, which were continually increased to match the rising demand of the growing plants. All plants were grown for approximately five weeks and then harvested.

Wheat plants grown in pots exhibited photosynthetic acclimation when supplied with low fixed

amounts of nitrogen, and elevated CO<sub>2</sub> exacerbated this effect. In contrast, hydroponically grown wheat that received gradually increasing nutrient supplies, which became ever-larger with increasing plant size, exhibited no signs of photosynthetic acclimation when grown at elevated CO<sub>2</sub> even at low nitrogen availability. These observations led the authors to conclude low nitrogen fertilization may not lead to photosynthetic acclimation in elevated CO<sub>2</sub>, as long as the nitrogen supply keeps pace with the relative growth rate of the plants. Consequently, it is important for researchers who use potted plants to increase plant nutrient supply in proportion to plant growth as their experiments progress, in order to avoid inducing photosynthetic acclimation via the dilution of tissue nitrogen contents that typically results from enhanced carbohydrate and biomass production in elevated CO<sub>2</sub>.

It appears plants with inherently large sink capacity have the ability to respond strongly and persistently to atmospheric CO<sub>2</sub> enrichment. In other plants, a short-term reduction in source strength often overcomes sink-induced limitations on growth responses to atmospheric CO<sub>2</sub> enrichment. Foliar reductions resulting from clipping events—or non-invasive reductions in source strength—nearly always eliminated CO<sub>2</sub>-induced photosynthetic acclimation. Moreover, acclimation to elevated CO<sub>2</sub> may actually be an indirect effect induced by insufficient amounts of soil nitrogen; if soil nitrogen content is too low, additional sink strength cannot be developed in some plants, which consequently allows carbohydrate accumulation in source leaves that can induce photosynthetic acclimation by feedback inhibition processes. As the CO<sub>2</sub> content of the air rises and increases plant photosynthetic rates, many plants will respond by reducing rubisco contents, which frees up large quantities of nitrogen that may be used to enhance sink strength to keep plants from exhibiting photosynthetic acclimation.

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## 2.2 Antioxidants

Oxidation is a chemical process that occurs naturally in plants, animals, and humans. Although the process is vital for life, it can produce free radicals, including reactive oxygen species (ROS), in a series of chain reactions that lead to cell damage and cell death. In humans, oxidative stress has been linked to cardiovascular disease, cancer, neurodegenerative disorders, and other chronic diseases. Nature's way of responding to the threats posed by such radicals is to neutralize and inhibit their reactions via complex systems of multiple types of antioxidants.

Plants, animals, and humans each harbor defense systems comprised of various types of antioxidants, including vitamin A, vitamin C, and vitamin E, and enzymes such as catalase, superoxide dismutase, and various peroxidases. Inadequate levels of antioxidants, or inhibition of antioxidant enzymes, can lead to oxidative stress.

The impact of rising atmospheric CO<sub>2</sub> on antioxidant compounds and enzymes found in plants is examined in Section 7.3.1.1 of Chapter 7, *Human Health*, this volume, where it is noted higher levels of atmospheric CO<sub>2</sub> tend to reduce oxidative stress, resulting in a reduction in antioxidant enzyme activity because fewer such enzymes are needed to counter the stress. As a result, plants are able to direct more of their limited resources into the production of other plant tissues or processes essential to their continued growth and development. In some cases, such resources are invested into the production and enhancement of antioxidative compounds, which are known to provide health benefits to animals and humans that ingest them.

### 2.3 Chlorophyll

- Atmospheric CO<sub>2</sub> enrichment may either increase, decrease, or have no effect on leaf chlorophyll concentration. Even when it is decreased, the reallocation of its nitrogen generally occurs without any adverse consequences, as most plants displaying this response typically continue to exhibit significant increases in photosynthesis and biomass production.

Plants of all types that are grown in elevated CO<sub>2</sub> environments often exhibit some degree of physiological adaptation. The most common of these is photosynthetic acclimation or down-regulation, which is characterized by reductions in the activity and/or amount of the primary plant carboxylating enzyme rubisco (see Section 2.1 Acclimation). In addition, plant acclimation to elevated atmospheric CO<sub>2</sub> may lead to reductions in leaf nitrogen and chlorophyll concentrations, the latter of which is discussed below.

Photosynthetic acclimation to elevated CO<sub>2</sub> is not a detrimental phenomenon, but rather a positive phenomenon that facilitates the redistribution of nitrogen and other important resources away from a plant's photosynthetic apparatus toward other sites where growth-limiting processes also need these resources. In an open-top chamber experiment on spruce saplings that lasted three years, for example, CO<sub>2</sub>-enriched trees exhibited photosynthetic rates 62% greater than those of their ambiently grown counterparts, in spite of their lower leaf chlorophyll contents (Centritto and Jarvis, 1999). Similar studies on herbaceous (Ong *et al.*, 1998; Pritchard *et al.*, 2000) and woody (Grams *et al.*, 1999; Ormrod *et al.*, 1999) species have yielded analogous results, indicating leaf chlorophyll concentrations in these species are often much greater than what are actually needed for adequate photosynthesis in CO<sub>2</sub>-enriched atmospheres.

Nevertheless, atmospheric CO<sub>2</sub> enrichment does not always result in decreased leaf chlorophyll concentrations. In an open-top chamber experiment with alfalfa, for example, plants grown at an atmospheric CO<sub>2</sub> concentration of 600 ppm had greater leaf chlorophyll concentrations than those observed in plants grown at 340 ppm (Sgherri *et al.*, 1998). And exposure of an orchid to a super-elevated CO<sub>2</sub> concentration of 10,000 ppm led to a 64% increase in leaf chlorophyll concentration relative to that measured in leaves of plants grown at ambient CO<sub>2</sub> (Gouk *et al.*, 1999).

In between these two responses, increases in the

air's CO<sub>2</sub> content sometimes have been demonstrated to have no significant effect on leaf chlorophyll concentration. Sicher and Bunce (1999), for example, reported twice-ambient CO<sub>2</sub> concentrations elicited no change in leaf chlorophyll contents of potato plants during a three-year study. Even with higher CO<sub>2</sub> enrichment levels (870 ppm above ambient concentrations), Monje and Bugbee (1998) did not detect any CO<sub>2</sub>-induced changes in leaf chlorophyll content of wheat. Similar results have been reported in woody plants, where a doubling of the atmospheric CO<sub>2</sub> concentration had no significant impact on leaf chlorophyll concentrations within sugar maple (Li *et al.*, 2000) and oak species (Carter *et al.*, 2000; Styliński *et al.*, 2000).

These studies demonstrate atmospheric CO<sub>2</sub> enrichment may either increase, decrease, or have no effect on leaf chlorophyll concentrations, and even when leaf chlorophyll concentrations are decreased the reallocation of the nitrogen that is essential for producing chlorophyll and other photosynthetic components typically occurs without any adverse consequences. Most plants displaying this response almost always continue to exhibit significant increases in photosynthesis and biomass production.

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## 2.4 Early Spring Growth

- Studies conducted on several tree species suggest either enhanced carbohydrates stored in terminal branchlets or nitrogen stored in second-year leaves may be remobilized to facilitate greater first-flush needle or leaf growth the following year as a result of atmospheric CO<sub>2</sub> enrichment.

Each year in the spring, when vegetation awakens from its winter dormancy and begins to grow, producing new flushes of leaves and needles, the vegetative demand for carbon is so great that plant photosynthesis actually draws down the atmosphere's CO<sub>2</sub> concentration in the Northern Hemisphere by several ppm (in the Southern Hemisphere too, but to a much lesser extent due to the larger surface area of oceans). In the fall, when much of the new biomass produced over the spring and summer begins to

senesce and decay, large amounts of carbon are returned to the atmosphere, causing the air's CO<sub>2</sub> concentration to rise by several ppm. Hence, an annual oscillation is imposed on the ever-increasing CO<sub>2</sub> content of the atmosphere in each hemisphere, in which the CO<sub>2</sub> concentration first decreases with spring and summer growth and then increases with fall senescence.

In analyzing this annual atmospheric CO<sub>2</sub> oscillation, several scientists have noted the spring drawdown of the air's CO<sub>2</sub> content is beginning a few days earlier than it did several decades ago. Some have attributed this phenomenon to CO<sub>2</sub>-induced global warming, but others have suggested the increasingly earlier occurrence of what may be called biological spring may result from an amplification of early spring branch growth that is provided by the ever-increasing aerial fertilization effect of the ongoing rise in the air's CO<sub>2</sub> content.

One of the first papers to anticipate this phenomenon was that of Lovelock *et al.* (1999), who enclosed branchlets of 30-m tall *Luehea seemannii* trees in open-top chambers suspended within the trees' upper canopies and fumigated them with air of either 360 or 750 ppm CO<sub>2</sub> for nearly 40 weeks in a study of the effects of elevated CO<sub>2</sub> on photosynthesis, growth, and reproduction in this deciduous tropical tree species. By these means they determined leaves of branchlets grown in CO<sub>2</sub>-enriched air displayed net photosynthetic rates approximately 30% greater than those of leaves of branchlets grown in ambient air. The additional carbohydrates produced by this phenomenon were not used by CO<sub>2</sub>-enriched branchlets to increase leaf growth or reproductive efforts. Instead, they were stored in terminal woody tissues. This observation led the authors to suggest enhanced carbohydrate storage in terminal branchlets may facilitate greater first-flush leaf growth the following year.

Documentation of this phenomenon was first provided by Idso *et al.* (2000), who periodically measured the lengths, dry weights, and leaf chlorophyll concentrations of new branches that each spring emerged from sour orange trees that had been growing out-of-doors in clear-plastic-wall open-top chambers for more than 10 years in air of either 400 or 700 ppm CO<sub>2</sub>. Although new spring branch growth always began on exactly the same day in both the ambient and CO<sub>2</sub>-enriched chambers, the initial rate of new-branch biomass production was vastly greater in the CO<sub>2</sub>-enriched trees. Three weeks after branch growth began, for example, new branches on the CO<sub>2</sub>-enriched trees were typically more than four times

more massive than their counterparts on the ambient-treatment trees, while on a per-tree basis, there was more than six times more new-branch biomass on the trees growing in the CO<sub>2</sub>-enriched chambers than in the ambient-air chambers. Just as rapidly as this ultra-enhancement of new branch growth began, however, it also declined, and 10 weeks into the growing season the CO<sub>2</sub>-enriched/ambient-treatment new-branch biomass ratio had leveled out at a value commensurate with seasonal standing biomass and fruit production ratios—just under a doubling.

Based on their experimentally observed results, Idso *et al.* calculated the length of time by which the new-branch dry weight of the CO<sub>2</sub>-enriched trees led that of the ambient-treatment trees over the first two months of the growing season, determining the 300-ppm increase in the air's CO<sub>2</sub> concentration caused the trees to begin the significant portion of their spring drawdown of atmospheric CO<sub>2</sub> fully two weeks earlier than what otherwise would have been normal. They calculated that for the 43-ppm increase in the air's CO<sub>2</sub> concentration experienced between 1960 and 1994, biological spring for sour orange trees should have occurred two days earlier in 1994 than it did in 1960.

By way of comparison, there was an approximate seven-day advancement in the time of occurrence of the declining phase of the atmosphere's seasonal CO<sub>2</sub> cycle over this time period, and surface reflectance measurements made by satellites revealed a similar advancement in the springtime "greening of the Earth" in high northern latitudes. Idso *et al.*'s data thus suggest a significant portion of this advancement may have been due to the dramatic stimulation of initial new-branch growth provided by the increase in the air's CO<sub>2</sub> concentration experienced over that time interval. But where did the CO<sub>2</sub>-enriched sour orange trees get the extra nitrogen needed to support the enhanced production of new branch and leaf tissue that occurred at the beginning of the growing season?

In an experiment bearing on this question, Idso *et al.* (2001) measured the concentrations of three soluble leaf proteins having molecular masses of 33, 31, and 21 kDa at weekly intervals for one full year in the foliage of the CO<sub>2</sub>-enriched and ambient-treatment trees. During the central portion of the year, the abundances of the three proteins were generally lower in the leaves of the CO<sub>2</sub>-enriched trees than they were in the leaves of the ambient-treatment trees; but in the latter part of the year and continuing for a short while into the next year, this relationship was reversed, as the leaf concentrations of the three

proteins in the CO<sub>2</sub>-enriched trees surpassed those of the proteins in the trees growing in ambient air. Then in the spring, when new growth began to appear, the concentrations of the three proteins in the foliage of the CO<sub>2</sub>-enriched trees plummeted, possibly as a result of giving up the nitrogen they had stored over winter to supply the needs of the newly developing branches and leaves.

According to Idso *et al.*, the N-terminal amino acid sequence of the 21-kDa protein has homology with sporamin B, an implicated vegetative storage protein (VSP) that can comprise 60 to 80% of the total soluble protein found in sweet potato tubers. They also determined it shares low sequence homology with trifoliatin, a soluble leaf protein from trifoliolate orange that shares 62% amino acid similarity with sporamin B and can comprise up to 65% of total leaf protein. In addition, they say "immunoelectron microscopy demonstrated the presence of the proteins within amorphous material in the vacuoles of mesophyll cells, where VSPs are commonly located."

Perhaps most telling, starting at about day-of-the-year 225, the CO<sub>2</sub>-enriched trees experienced a period of leaf senescence and abscission that was not observed in the ambient-treatment trees. "This phenomenon," Idso *et al.* report, "peaked at about day 300, when the CO<sub>2</sub>-enriched trees shed leaves at a rate 2.5–2.7 times greater than the normal background rate of the ambient-treatment trees, whereupon the enriched-tree leaf-fall rate diminished, returning to normal by the end of the year."

According to the hypothesis formulated by Idso *et al.*, nitrogen reabsorbed from these second-year leaves during the process of senescence became available for storage in first-year leaves of the CO<sub>2</sub>-enriched trees, going into the 21-kDa protein starting at about day 225, into the 31-kDa protein starting at about day 265, and into the 33-kDa protein starting at about day 335, according to the trends defined by their weekly measurements of leaf protein concentrations. Then, when new branches and leaves began appearing in the spring, the stored nitrogen was remobilized from the prior first-year leaves (which now became second-year leaves) to supply the needs of the developing branch and leaf tissues, whereupon the concentrations of the three putative storage proteins in the now-second-year CO<sub>2</sub>-enriched leaves rapidly dropped to levels once again less than those observed in similar-age ambient-treatment leaves, and they remained at that reduced level until the second-year leaves gave up even more nitrogen prior to their senescence in the fall.

Other experimental evidence for a CO<sub>2</sub>-induced

stimulation of new spring branch growth has been provided by Olszyk *et al.* (2001), who grew Ponderosa pine seedlings out-of-doors for two years in controlled-environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of 390 and 670 ppm in combination with low (40 ppb) and high (60 ppb) ozone concentrations. They found the elevated CO<sub>2</sub> enhanced mean annual rates of net photosynthesis by 39% and increased bud lengths at the end of the second growing season in the low ozone treatment by 17%, which led to a transitory stimulation (+38%) of elongation and growth of terminal buds the following spring.

That super-stimulation of initial perennial plant growth in the early spring may be a ubiquitous phenomenon is suggested by the research of Bushway and Pritts (2002). They grew over-wintering strawberry plants in controlled environment chambers maintained at ambient (375 ppm) and elevated (700 to 1,000 ppm) atmospheric CO<sub>2</sub> concentrations until new blooms began to form on plants. They then moved the plants to a common greenhouse maintained at the ambient CO<sub>2</sub> concentration. The extra CO<sub>2</sub> stimulated rates of photosynthesis in the leaves of the over-wintering strawberry plants by more than 50%, which led to greater amounts of starch being found in key plant organs when new spring growth began. Plants grown in elevated CO<sub>2</sub> had two-, three-, and four-times the amount of starch in their crowns, leaves, and roots, respectively, than their ambiently grown counterparts. In addition, plants grown in elevated CO<sub>2</sub> flowered and fruited an average of four and seven days earlier than plants grown in ambient air, respectively.

Lim *et al.* (2004) correlated the monthly rate of relative change in normalized difference vegetation index (NDVI), which they derived from advanced very-high-resolution radiometer data, with the rate of change in atmospheric CO<sub>2</sub> concentration ( $\delta$ CO<sub>2</sub>) during the natural growing season within three eco-region zones of North America (the Arctic and Sub-Arctic Zone, the Humid Temperate Zone, and the Dry and Desert Zone, which they further subdivided into 17 regions) over the period 1982–1992. They explored the temporal progression of annual minimum NDVI over the period 1982–2001 throughout the eastern humid temperate zone of North America. In all 17 regions but one, “ $\delta$ CO<sub>2</sub> was positively correlated with the rate of change in vegetation greenness in the following month, and most correlations were high,” which they state is consistent with a CO<sub>2</sub> fertilization effect of the type observed in experimental manipulations of the air’s CO<sub>2</sub> content

that report a stimulation of photosynthesis and above-ground production at high CO<sub>2</sub>. In addition, and most importantly, they determined the yearly “minimum vegetation greenness increased over the period 1982–2001 for all the regions of the eastern humid temperate zone in North America.”

Lim *et al.* opine rising CO<sub>2</sub> could “increase minimum greenness by stimulating photosynthesis at the beginning of the growing season,” citing Idso *et al.* (2000). Hence, by looking for a manifestation of the CO<sub>2</sub> fertilization effect at the time of year it is apt to be most strongly expressed, Lim *et al.* may have found it. Between 1982 and 2001, for example, the air’s CO<sub>2</sub> concentration rose by approximately 30 ppm. From Idso *et al.*’s findings of more than a 300% initial increase in the biomass of new sour orange tree branches for a 300-ppm increase in the air’s CO<sub>2</sub> concentration and more than a 500% initial increase in per-tree new-branch biomass, it can be calculated that yearly minimum greenness should have increased by a value between just over 30% and just over 50%, if other woody plants respond to atmospheric CO<sub>2</sub> enrichment as sour orange trees do. When the mean 19-year increase in NDVI for the seven regions for which Lim *et al.* present data is calculated, an increase of just over 40% is seen, indicating Lim *et al.*’s data are not only qualitatively consistent with the hypothesis of CO<sub>2</sub>-induced ultra-stimulation of early spring branch growth, but quantitatively as well.

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## 2.5 Flowers

- At higher atmospheric CO<sub>2</sub> concentrations, plants often produce more and larger flowers, as well as a number of other flower-related changes having significant implications for plant productivity and survival, almost all of which are positive.

In one of the earliest papers to address the subject of atmospheric CO<sub>2</sub> enrichment effects on flowers, Idso *et al.* (1990) grew water lilies in sunken metal stock tanks located out-of-doors and enclosed within clear-plastic-wall open-top chambers through which air of either 350 or 650 ppm CO<sub>2</sub> was continuously circulated. Over the course of two growing seasons, he and his colleagues measured plant responses to these two environmental treatments. They found the water lilies in the CO<sub>2</sub>-enriched enclosures grew better than the water lilies in the ambient CO<sub>2</sub> enclosures: the leaves in the CO<sub>2</sub>-enriched tanks were larger and more substantial, and 75% more of them were produced over the course of the initial five-month growing season.

Each of the plants in the CO<sub>2</sub>-enriched tanks also produced twice as many flowers as the plants growing in normal air, and the flowers that blossomed in the CO<sub>2</sub>-enriched air were more substantial than those that bloomed in the air of normal CO<sub>2</sub> concentration: they had more petals, the petals were longer, they had a greater percentage of dry matter content, and each flower weighed about 50% more. In addition, the stems that supported the flowers were slightly longer in the CO<sub>2</sub>-enriched tanks, and the percentage of dry matter contents of both the flower and leaf stems were greater, so the total dry matter in the flower and leaf stems in the CO<sub>2</sub>-enriched tanks exceeded that of the flower and leaf stems in the ambient-air tanks by approximately 60%.

Deng and Woodward (1998) studied the direct and interactive effects of elevated CO<sub>2</sub> and nitrogen supply on strawberries by growing them in controlled-environment glasshouses maintained at

atmospheric CO<sub>2</sub> concentrations of either 390 or 560 ppm at three levels of nitrogen for nearly three months. They found strawberries growing at the elevated CO<sub>2</sub> concentration contained additional sugar and physical mass that enabled them to support significantly greater numbers of flowers and fruits than strawberry plants growing at 390 ppm CO<sub>2</sub>. This effect ultimately led to total fresh fruit weights 42 and 17% greater in CO<sub>2</sub>-enriched plants maintained at the highest and lowest nitrogen levels, respectively.

Lake and Hughes (1999) found a 380-ppm increase in the air's CO<sub>2</sub> content elicited a 35% increase in the total plant biomass of nasturtiums (*Tropaeolum majus*). Although it did not affect flower size in this species, the total flower nectar volume produced by the CO<sub>2</sub>-enriched nasturtiums was 2.4-fold greater than that produced by ambient-grown control plants. Likewise, Dag and Eisikowitch (2000) reported atmospheric CO<sub>2</sub> enrichment up to 1,000 ppm doubled both the average nectar volume and sugar production per flower in greenhouse-grown melons (*Cucumis melo*).

Johnson and Lincoln (2000) report an annual plant native to the southeastern USA (*Heterotheca subaxillaris*) increased its total biomass by 20% in response to a 300-ppm increase in the air's CO<sub>2</sub> content. In addition, the elevated CO<sub>2</sub> increased reproductive flower biomass and induced flowering much earlier in the CO<sub>2</sub>-enriched plants than in the ambient-air-grown plants. And in another experiment, Niu *et al.* (2000) found yellow and primrose pansies (*Viola x wittrockiana*) increased their total dry weights by 10 to 30% in response to a 600-ppm increase in the CO<sub>2</sub> content of the air, and atmospheric CO<sub>2</sub> enrichment increased flower size by 4 to 10%.

Carvalho and Heuvelink (2001) report atmospheric CO<sub>2</sub> enrichment positively influences several external quality characteristics of chrysanthemums, including increasing the plant's stem length, number of lateral branches, number of flowers, and size of flowers. Aloni *et al.* (2001) found a 450-ppm increase in the air's CO<sub>2</sub> content completely ameliorated a 75% high-temperature-induced reduction in bell pepper (*Capsicum annuum* L.) pollen production observed under ambient CO<sub>2</sub> concentrations. In addition, although high temperature reduced the number of seeds produced per fruit in ambient-grown plants by 68%, it reduced this parameter only by 9% in CO<sub>2</sub>-enriched plants.

Deckmyn *et al.* (2001) grew white clover plants (*Trifolium repens* L., cv. Mervi) in four small greenhouses, two of which allowed 88% of the

incoming UV-B radiation to pass through their roofs and walls, and two of which allowed 82% to pass through. One of the two greenhouses in each of the UV-B treatments was maintained at ambient CO<sub>2</sub> (371 ppm), and the other was maintained at elevated CO<sub>2</sub> (521 ppm). Midway through the four-month summer growing season, flower numbers were counted, revealing the 40% increase in atmospheric CO<sub>2</sub> concentration stimulated the production of flowers in the low UV-B treatment by 22% and in the slightly higher UV-B treatment by 43%.

By 2002, so many scientists had weighed in on the subject that Jablonski *et al.* (2002) conducted a meta-analysis of 159 peer-reviewed scientific journal articles published between 1983 and 2000 dealing with the effects of atmospheric CO<sub>2</sub> enrichment on the reproductive growth characteristics of several domesticated and wild plants. In calculating the mean responses reported in those papers, Jablonski *et al.* found for increases in the air's CO<sub>2</sub> concentration ranging from approximately 150 to 450 ppm (rough average of 300 ppm), across all species studied, the extra CO<sub>2</sub> supplied to the plants resulted in 19% more flowers, 18% more fruits, 16% more seeds, 4% greater individual seed mass, 25% greater total seed mass (equivalent to yield), and 31% greater total mass.

More studies demonstrating similar positive effects of atmospheric CO<sub>2</sub> enrichment on flowering characteristics followed. Silberbush *et al.* (2003), for example, grew small and large bulbs of *Hippeastrum* (which produces amaryllis flowers) in greenhouses receiving atmospheric CO<sub>2</sub> concentrations of 350 and 1000 ppm for about four hours of each day for 233 days with different combinations of nitrogen and potassium fertilization, in order to study the interactive effects of these parameters on bulb size. They found elevated CO<sub>2</sub> consistently increased bulb size across all nitrogen and potassium concentrations, with initially larger bulbs yielding the greatest size of final bulbs. On a percentage basis, smaller bulbs were slightly more responsive to atmospheric CO<sub>2</sub> enrichment than were larger bulbs. Under optimal nitrogen and potassium fertilization, the 650-ppm increase in the air's CO<sub>2</sub> concentration increased the size of smaller and larger bulbs by about 18 and 14%, respectively, suggesting as the CO<sub>2</sub> content of the air increases, *Hippeastrum* bulbs will increase their size, thus leading to enhanced bulb quality and flower (amaryllis) production.

Palacios and Zimmerman (2007) piped flue gas generated by the Duke Energy-North America Power Plant at Moss Landing, California (USA)

approximately 1 km to a site where it was bubbled through outdoor flow-through seawater aquaria at rates that produced four different aqueous CO<sub>2</sub> treatments characteristic of “(1) the present day atmosphere, with approximately 16 μM CO<sub>2</sub>(aq), (2) CO<sub>2</sub> projected for 2100 that increases the CO<sub>2</sub>(aq) concentration of seawater to approximately 36 μM CO<sub>2</sub>(aq), (3) CO<sub>2</sub> projected for 2200 that increases the CO<sub>2</sub>(aq) concentration of seawater to 85 μM CO<sub>2</sub>(aq), and (4) a dissolved aqueous CO<sub>2</sub> concentration of 1123 μM CO<sub>2</sub>(aq),” which in the earlier study of Zimmerman *et al.* (1997) had tripled the light-saturated photosynthesis rate of the eelgrass they had studied.

In their newer study, the researchers report the elevated CO<sub>2</sub> “led to significantly higher reproductive output, below-ground biomass and vegetative proliferation of new shoots in light-replete treatments,” i.e., those receiving light at 33% of the surface irradiance level. More specifically, they found “shoots growing at 36 μM CO<sub>2</sub>(aq) were 25% larger than those in the unenriched treatment [16 μM CO<sub>2</sub>(aq)],” and “at 85 μM CO<sub>2</sub>(aq) shoots were 50% larger than those in the unenriched treatment and at 1123 μM CO<sub>2</sub>(aq) shoots were almost twice as large as those in the unenriched treatment.” In addition, they found, at 1123 μM CO<sub>2</sub>(aq) “22% of the shoots differentiated into flowers, more than twice the flowering output of the other treatments at this light level.”

These findings have far-reaching implications. Noting “increased CO<sub>2</sub>(aq) is capable of increasing eelgrass reproductive output via flowering, and area-specific productivity via vegetative shoot proliferation under naturally replete light regimes,” Palacios and Zimmerman state, “the resulting increases in eelgrass meadow density may initiate a positive feedback loop that facilitates the trapping of sediments and prevents their resuspension, thereby reducing turbidity and increasing light penetration in coastal habitats,” such that the resulting increased light penetration “may allow seagrass colonization depths to increase even further.”

The two researchers also suggest the CO<sub>2</sub>-induced increase in the productivity of eelgrass may “enhance fish and invertebrate stocks as well.” They go so far as to suggest the “deliberate injection of CO<sub>2</sub> to seawater may facilitate restoration efforts by improving the survival rates of recently transplanted eelgrass shoots,” noting “it can buffer the negative effects of transplant shock by increasing rhizome reserve capacity and promoting shoot proliferation in light-replete environments.” In addition, they say it

“may also facilitate eelgrass survival in environments where conditions are periodically limiting, such as long dark winters or unusually warm summers that produce unfavorable productivity to respiration ratios,” and they write, “CO<sub>2</sub> injection may also promote flowering and seed production necessary for expansion and maintenance of healthy eelgrass meadows.” Finally, they suggest, “rising concentrations of CO<sub>2</sub>(aq) may increase vegetative propagation and seed production of other seagrass populations besides eelgrass.”

In another review paper, Springer and Ward (2007) summarize “the results of 60 studies reporting flowering-time responses (defined as the time to first visible flower) of both crop and wild species at elevated CO<sub>2</sub>.” They found “all possible responses have been observed both among species as well as within species, including accelerated, delayed and no change in flowering time in response to elevated CO<sub>2</sub>.” However, they write, “flowering-time responses of wild species grown at elevated CO<sub>2</sub> are much more evenly distributed, in that a similar number of studies report accelerated, delayed, or no change in flowering time, whereas crops primarily showed accelerated flowering (approx. 80% exhibited accelerated flowering).” They also note, “plants utilizing both the C<sub>3</sub> and C<sub>4</sub> photosynthetic pathways show altered flowering time with elevated CO<sub>2</sub>,” but “two crop species that account for a substantial portion of the world’s agricultural production, soybean [a C<sub>3</sub> crop] and maize [a C<sub>4</sub> crop], do not show consistent patterns in the response of flowering time at elevated CO<sub>2</sub>.”

Springer and Ward additionally determined “studies performed within a genus also show a lack of consistent flowering-time response to elevated CO<sub>2</sub>,” and among only 10 genotypes of a single well-studied species (*Arabidopsis thaliana*), “all possible flowering-time responses to elevated CO<sub>2</sub>, including delayed, accelerated and unaltered flowering times, were observed.” Finally, they report, “a majority of multifactor studies that measured flowering time report no interaction between elevated CO<sub>2</sub> and other environmental factors, such as temperature, ... nutrient availability, ... light, ... and ozone,” although “a limited number of elevated CO<sub>2</sub> studies do show significant interactive effects with other environmental factors.”

Springer and Ward thus conclude the studies they reviewed “clearly show that future increases in atmospheric CO<sub>2</sub> will have major effects on the flowering time of both wild and crop species,” but “at this time it is not possible to account for the wide

variation in flowering-time responses because knowledge of the underlying physiological and molecular mechanisms is incomplete.”

Darbah *et al.* (2008) studied the effects of long-term exposure of birch (*Betula papyrifera*) trees to elevated CO<sub>2</sub> (an extra 200 ppm) on flower and pollen production at the Aspen FACE site in Rhinelander, Wisconsin (USA) in the eighth and ninth years (2006 and 2007) of that experiment. They found “an increase of 140% and 70% for 2006 and 2007, respectively, in the total number of trees that produced male flowers under elevated CO<sub>2</sub> and an increase of 260% in 2006 and 100% in 2007, respectively, in the quantity of male flowers produced under elevated CO<sub>2</sub>.”

According to the six scientists, “the increases in the number of trees and in the quantity of male flowers produced under elevated CO<sub>2</sub> implies that more birch pollen will be produced.” They note, “these results support the findings of Curtis *et al.* (1994, 1996), Johnson and Lincoln (2000), Edwards *et al.* (2001), Jablonski *et al.* (2002), Bunce (2005) and Ladeau and Clark (2006a,b), which were that elevated CO<sub>2</sub> increases reproductive potential through increased pollination, and hence, fertilization and viable seed formation,” in harmony with the hypothesis of Herms and Mattson (1992), and “birch trees under adequate carbohydrate status [such as provided by atmospheric CO<sub>2</sub> enrichment] tend to favor male flower production.” They conclude by noting, “since sexual reproductive development is an important stage in the life cycle of plants, any change in the processes involved might have significant implications for the productivity of the plants and their survival.” The implications in the case of birch trees and atmospheric CO<sub>2</sub> enrichment clearly would be positive.

Johnston and Reekie (2008) write, “there have been marked changes in plant phenology over the past century” and these changes “have been interpreted as a consequence of the increase in temperature that has been observed over this time.” In addition, they speculate, “the concentration of atmospheric CO<sub>2</sub> may also directly affect time of flowering, even in the absence of temperature change.” They examined the effects of elevated atmospheric CO<sub>2</sub> concentration by itself (ambient and ambient + 330 ppm) and the combined effect of elevated CO<sub>2</sub> and elevated air temperature (ambient + 1.5°C) on the flowering phenology of 22 species of plants in the family Asteraceae, which were grown under natural seasonally varying temperature and day length in separate compartments of a glasshouse in Wolfville,

Nova Scotia, Canada.

They found, “on average, elevated CO<sub>2</sub> by itself advanced flowering by four days” and “increasing temperature as well as CO<sub>2</sub> advanced flowering by an additional three days.” They also found “CO<sub>2</sub> was more likely to hasten phenology in long- than in short-day species” and “early- and late-flowering species did not differ in response to elevated CO<sub>2</sub>, but the combined effect of elevated CO<sub>2</sub> and temperature hastened flowering more in early- than late-flowering species.” Johnston and Reekie conclude, “the direct effect of CO<sub>2</sub> on phenology may be as important as its indirect effect through climate change.”

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## 2.6 Fluctuating Asymmetry

- Higher concentrations of atmospheric CO<sub>2</sub> tend to reduce fluctuating asymmetry in plant leaves, leading to more symmetrical leaves that appear to be less susceptible to attack by herbivores.

Fluctuating asymmetry (FA) is the terminology used to describe small variations from perfect symmetry in otherwise bilaterally symmetrical characters in an organism (Moller and Swaddle, 1997). It is believed to arise in consequence of developmental instability experienced during ontogeny that is caused by various stresses, including both genetic and environmental factors (Martel *et al.*, 1999; Cornelissen and Stiling, 2005); it has been studied extensively in animals but less so in plants (Moller and Shykoff, 1999).

In the first study to address the effects of atmospheric CO<sub>2</sub> enrichment on leaf asymmetry and how herbivores respond to these effects, Cornelissen *et al.* (2004) opened up a new window through which to view the potential effects of the ongoing rise in the air's CO<sub>2</sub> content on the plant and animal components of the biosphere. They conducted their study on a native scrub-oak community at the Kennedy Space Center, Titusville, Florida (USA), which is dominated by myrtle oak (*Quercus myrtifolia*) and sand live oak (*Quercus geminata*), under atmospheric CO<sub>2</sub> concentrations of approximately 370 and 700 ppm. Based on measurements of distances from the leaf midrib to the left and right edges of the leaf at its widest point and leaf areas on the left and right sides of the leaf midrib, Cornelissen *et al.* determined “asymmetric leaves were less frequent in elevated CO<sub>2</sub>, and, when encountered, they were less asymmetric than leaves growing under ambient CO<sub>2</sub>.” In addition, they found “*Q. myrtifolia* leaves under elevated CO<sub>2</sub> were 15.0% larger than in ambient CO<sub>2</sub> and *Q. geminata* leaves were 38.0% larger in elevated CO<sub>2</sub> conditions.” They also determined “elevated CO<sub>2</sub> significantly increased tannin concentration for both *Q. myrtifolia* and *Q. geminata* leaves” and “asymmetric leaves contained significantly lower concentrations of tannins than symmetric leaves for both *Q. geminata* and *Q. myrtifolia*.”

Commenting on their primary findings of reduced

percentages of leaves experiencing asymmetry in the presence of elevated levels of atmospheric CO<sub>2</sub> and the lesser degree of asymmetry exhibited by affected leaves in the elevated CO<sub>2</sub> treatment, Cornelissen *et al.* state, “a possible explanation for this pattern is the fact that, in contrast to other environmental stresses, which can cause negative effects on plant growth, the predominant effect of elevated CO<sub>2</sub> on plants is to promote growth with consequent reallocation of resources (Docherty *et al.*, 1996).” Another possibility they discuss is “the fact that CO<sub>2</sub> acts as a plant fertilizer” and, as a result, “elevated CO<sub>2</sub> ameliorates plant stress compared with ambient levels of CO<sub>2</sub>,” which is one of the well-documented biological benefits of atmospheric CO<sub>2</sub> enrichment (Idso and Idso, 1994).

With respect to the ancillary finding of CO<sub>2</sub>-induced increases in tannin concentrations in the leaves of both oaks (a mean increase of approximately 35% for *Q. myrtifolia* and 43% for *Q. geminata*), it should be noted this phenomenon may provide protection against herbivores, and part of that protection may be associated with the observed CO<sub>2</sub>-induced reductions in the amount and degree of asymmetry in the leaves of the CO<sub>2</sub>-enriched trees. Consistent with this hypothesis, for example, Stiling *et al.* (1999, 2003) found higher abundances of leaf miners in the leaves of the trees in the ambient CO<sub>2</sub> chambers, where asymmetric leaves were more abundant, while in the more recent study it was determined leaf miners attacked asymmetric leaves more frequently than would be expected by chance alone in both CO<sub>2</sub> treatments.

In further support of this CO<sub>2</sub>-induced benefit, Cornelissen and Stiling (2005) evaluated patterns of asymmetry in 40 leaves from each of 30 trees of each of two species of oak—sand live oak (*Quercus geminata*) and turkey oak (*Q. laevis*)—at the University of South Florida Botanical Garden in Tampa, Florida (USA), well before any herbivores had begun to attack the trees that growing season. Thereafter, patterns of leaf asymmetry, leaf quality, and herbivory were examined for 30 individual trees of each of the two oak species from March to October of the same year.

These “before and after” measurements clearly indicated differential herbivory patterns neither caused nor affected patterns of leaf FA. However, the two scientists suggest “herbivores may use asymmetry as a cue to plant quality and suitable oviposition sites,” as plants with a higher percentage of asymmetric leaves were attacked more frequently by various leaf miners, as were leaves on the same

plant that were more asymmetric. Cornelissen and Stiling report, “asymmetric leaves of both plant species exhibited better nutritional quality for herbivores than symmetric leaves,” with asymmetric leaves possessing “significantly lower concentrations of tannins [-22% for *Q. geminata* and -36% for *Q. laevis*] and higher nitrogen content [+8% for both species].”

Kaligaris *et al.* (2008) measured the degree of FA in “undamaged (not grazed, not visibly attacked by herbivores or pathogens) fully developed leaves” of the Mediterranean shrub *Myrtus communis* L. growing along an atmospheric CO<sub>2</sub> gradient (570, 530, 490, 450, 410, and 370 ppm) moving away from a natural CO<sub>2</sub> spring “I Borboi” near Lajatico (Pisa, Tuscany, Italy) at distances of 2, 18, 34, 50, 66, and 82 m, respectively, from the CO<sub>2</sub> source. They found “a significant and negative correlation between CO<sub>2</sub> concentration and leaf FA,” such that “with increased CO<sub>2</sub> concentration the leaf FA decreased.” This result, they write, “confirms what was obtained by Cornelissen *et al.* (2004) on *Quercus myrtifolia* and *Quercus geminata* (in a short-term experiment).” In addition, they note, “*Myrtus communis*, grown under elevated CO<sub>2</sub> concentration at ‘I Borboi,’ showed a reduction in xylem embolism and an increase in hydraulic efficiency (Tognetti *et al.*, 2001),” stating “improved water relations could represent a good explanation for the observed reduction in leaf FA [as the air’s CO<sub>2</sub> content increased].”

Kaligaris *et al.* suggest “adaptation and selection could explain the tendency towards decreased leaf FA in plants from the CO<sub>2</sub> spring relative to ambient conditions,” since “the more symmetrical leaves under long-term elevated CO<sub>2</sub> concentration were more developmentally stable in these conditions.”

Consequently, and in light of the results discussed above, a reduction in leaf FA can be added to the ever-growing number of benefits plants likely will experience as the atmosphere’s CO<sub>2</sub> concentration continues to rise.

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## 2.7 Hormones

- Atmospheric CO<sub>2</sub> enrichment enhances plant growth, development, and ultimate yield (in the case of agricultural crops) by increasing the concentrations of plant hormones that stimulate cell division, cell elongation, and protein synthesis.

Investigating the effects of atmospheric CO<sub>2</sub> enrichment on plant hormones, Teng *et al.* (2006) grew well-watered and -fertilized thale cress (*Arabidopsis thaliana* (L.) Heynh.) plants from seed

to commencement of bolting in pots within controlled-environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 370 or 700 ppm, measuring several plant properties and processes. The elevated CO<sub>2</sub> increased the biomass production of the plants by 29% and leaf total nonstructural carbohydrates by 76%. With respect to plant hormones, it “significantly increased the IAA [indole-3-acetic acid, by 13.7%], GA3 [gibberellic acid, by 55.4%], ZR [zeatin riboside, by 15.6%], DHZR [dihydrozeatin ribosidem, by 55.9%] and iPA [isopentenyladenosine, by 74.6%] contents of leaves, but ... significantly reduced the ABA [abscisic acid, by 15.2%] content.”

The six Chinese researchers conclude by noting plant hormones “can enhance plant growth and development by stimulating cell division, cell elongation and protein synthesis (Yong *et al.*, 2000), whereas ABA is considered an inhibitor of leaf growth (Zhang and Davies, 1990).” In addition, they note, “plant hormone metabolism is dependent on the supply of carbohydrates (Taiz and Zeiger, 1998).” They conclude, “higher carbohydrate production,” such as that induced by atmospheric CO<sub>2</sub> enrichment, “may result in higher hormone concentrations, which in turn may enhance plant growth.” That phenomenon also was observed by Jitla *et al.* (1997) and Li *et al.* (2002), with the latter reporting elevated CO<sub>2</sub> increased the concentrations of several plant hormones in leaf and aerial root tips of an epiphytic CAM orchid by as much as 21-fold.

In another experiment, Li *et al.* (2009) grew six-year-old *Ginkgo biloba* tree saplings in pots containing fertile soil out-of-doors in open-top chambers maintained at either 350 or 700 ppm CO<sub>2</sub> at the Shenyang Arboretum of the Chinese Academy of Science in Shenyang, Liaoning Province (China) from 1 June to 30 September. In addition to measuring the growth of the trees’ terminal shoots over this period, they also measured leaf concentrations of the endogenous plant-growth regulator indole-3-acetic acid (IAA). After 40 days’ exposure to elevated CO<sub>2</sub>, the IAA concentration in the plants’ leaves was increased significantly relative to that of plants in the control treatment, and maximum IAA enhancement was observed 100 days after exposure, when leaf concentrations of IAA were fully 90% greater in the elevated CO<sub>2</sub> treatment. The growth increment of ginkgo terminal shoots in the high CO<sub>2</sub> chambers was increased by 44% from 20 days to 90 days of exposure to the elevated CO<sub>2</sub> conditions.

Jiang *et al.* (2012) write, “brassinosteroids (BRs) are a family of over 40 naturally occurring plant

steroid hormones that are ubiquitously distributed in the plant kingdom,” citing Clouse and Sasse (1998), Bishop and Koncz (2002), Krishna (2003), and Montoya *et al.* (2005). They report, “BRs play prominent roles in various physiological processes including the induction of a broad spectrum of cellular responses, such as stem elongation, pollen tube growth, xylem differentiation, leaf epinasty, root inhibition, induction of ethylene biosynthesis, proton pump activation, regulation of gene expression and photosynthesis, and adaptive responses to environmental stress,” citing Clouse and Sasse (1998), Dhaubhadel *et al.* (1999), Khripach *et al.* (2000), Krishna (2003), and Yu *et al.* (2004). They also note, “as potent plant growth regulators, BRs are now widely used to enhance plant growth and yield of important agricultural crops,” citing Khripach *et al.* (2000) and Divi and Krishna (2009).

The six scientists conducted an experiment to determine the effects of increased atmospheric CO<sub>2</sub> on such of these plant steroid hormones as are found in young cucumber plants, growing them at atmospheric CO<sub>2</sub> concentrations of either 380 (ambient) or 760 (enriched) ppm, and with or without being sprayed with a solution of brassinosteroids (0.1 μM 24-epibrassinolide).

Jiang *et al.* determined their doubling of the atmosphere’s CO<sub>2</sub> concentration resulted in a 44.1% increase in CO<sub>2</sub> assimilation rate. They state the BR treatment “also significantly increased CO<sub>2</sub> assimilation under ambient atmospheric CO<sub>2</sub> conditions” and “the increase was close to that by CO<sub>2</sub> enrichment.” Most interesting, they found the combined treatment of “plants with BR application under CO<sub>2</sub>-enriched conditions showed the highest CO<sub>2</sub> assimilation rate, which was increased by 77.2% relative to the control.” Likewise, they found “an elevation in the atmospheric CO<sub>2</sub> level from 380 to 760 ppm resulted in 20.5% and 16.0% increases in leaf area and shoot biomass accumulation, respectively,” and the plants that received the BR application “exhibited 22.6% and 20.6% increases in leaf area and shoot biomass accumulation, respectively.” The combined treatment of “CO<sub>2</sub> enrichment and BR application further improved the plant growth, resulting in 49.0% and 40.2% increases in leaf area and shoot biomass, relative to that of the control, respectively.”

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## 2.8 Glomalin

- Atmospheric CO<sub>2</sub> enrichment increases the production of glomalin, a protein created by fungi living in symbiotic association with the roots of 80% of the planet's vascular plants, which is being released to almost every soil in the world in ever-greater quantities with the passage of time, where it is having a positive impact on the biosphere.

In a multifaceted research program carried out at experimental sites in northern and southern California, USA, Rillig *et al.* (1999) studied belowground ecosystem responses to elevated atmospheric CO<sub>2</sub> concentrations over a period of several years, focusing their attention on arbuscular mycorrhizal fungi (AMF) that form symbiotic associations with plant roots. In addition, they measured soil concentrations of an AMF-produced glycoprotein called glomalin and evaluated its response to elevated CO<sub>2</sub>. They then studied the impact of glomalin on the formation of small soil aggregates and their subsequent stability.

The degree of soil aggregation and the stability of soil aggregates across many different soil types is closely related to the amount of glomalin in the soil. Rillig *et al.* wanted to see if the aboveground benefits of atmospheric CO<sub>2</sub> enrichment would trickle down, so to speak, from plant leaves to plant roots to symbiotic soil fungi to glomalin production to soil aggregate formation and, ultimately, to an enhanced stability of soil aggregates in the presence of water.

The researchers found the amount of fungal-produced glomalin in the soils of the CO<sub>2</sub>-enriched treatments in all three of the ecosystems they studied

was greater than that observed in the soils of corresponding ambient CO<sub>2</sub> treatments. They also observed increases in the mass of small soil aggregates in the treatments exposed to elevated CO<sub>2</sub>, and the stability of the small soil aggregates in the CO<sub>2</sub>-enriched treatments was greater than the stability of the aggregates in the ambient CO<sub>2</sub> treatments. And in one of their studies, where six CO<sub>2</sub> concentrations ranging from 250 to 750 ppm were imposed as treatments, they found “the proportion of soil mass in aggregates of 0.25–1 mm showed a linear increase along the CO<sub>2</sub> gradient,” and “glomalin concentrations followed a pattern similar to that of the small aggregate size class,” indicating increasing soil structure benefits with increasing concentrations of atmospheric CO<sub>2</sub>.

In a subsequent study conducted in New Zealand, Rillig *et al.* (2000) examined several characteristics of AMF associated with the roots of plants that had been growing for at least 20 years along a natural CO<sub>2</sub> gradient near a CO<sub>2</sub>-emitting spring. They found the elevated CO<sub>2</sub> significantly increased percent of root colonization by AMF in a linear fashion—and nearly fourfold—in going from 370 to 670 ppm. In addition, fungal hyphal length experienced a linear increase of more than threefold along the same CO<sub>2</sub> gradient, and total soil glomalin experienced a linear increase of approximately fivefold.

Just as more and longer roots help plants hold soil together and prevent its erosion, so too do more and longer fungal hyphae protect soil from disruption and dispersion. In addition, fungal-produced glomalin acts like a biological glue, helping to bind tiny particles of soil into small aggregates that are much more difficult to break down and blow or wash away. To have soil glomalin concentrations increase fivefold as a consequence of less than a doubling of the air’s CO<sub>2</sub> content is an astounding benefit.

Ross *et al.* (2000) measured soil carbon (C) and nitrogen (N) contents in areas exposed to atmospheric CO<sub>2</sub> concentrations on the order of 440 to 460 ppm and other areas exposed to concentrations on the order of 510 to 900 ppm, near a natural CO<sub>2</sub> vent in New Zealand. Their work bears on the question whether CO<sub>2</sub>-induced increases in soil-stabilizing fungal activities might lead to increases in soil carbon sequestration.

They found several decades of differential atmospheric CO<sub>2</sub> exposure had increased soil organic C and total N contents by approximately 24% each, and it had increased microbial C and N contents by more than 100% each. Hence, the scientists write, “storage of C and N can increase under prolonged

exposure to elevated CO<sub>2</sub>.” In addition, they conclude increased storage of soil organic matter can occur “even when soil C concentrations are already high,” as they were in the situation they investigated. Consequently, not only will the capacity of soils to store carbon increase because of the increasing aerial fertilization effect of atmospheric CO<sub>2</sub> enrichment—which enhances plant growth and results in more carbon being transferred to the soil—increasingly active soil fungi will help to keep ever-greater portions of that carbon better preserved in increasingly more stable soils.

Augmented soil carbon sequestration is but the beginning of benefits that can be expected from CO<sub>2</sub>-enhanced AMF growth and glomalin production. Rillig *et al.* (2001) report the results of a FACE study of sorghum they conducted near Phoenix, Arizona (USA), where they found an approximate 50% increase in the air’s CO<sub>2</sub> content increased fungal hyphae lengths by 120% and 240% in wet and dry irrigation treatments, respectively, with the mass of water-stable soil aggregates increasing by 40% and 20% in the same respective treatments. They note, “soil structure and water-stable aggregation are crucial for facilitating water infiltration, soil-borne aspects of biogeochemical cycling processes, success of sustainable agriculture, and for providing resistance against erosional loss of soil (Oades, 1984; Elliott and Coleman, 1988; Van Veen and Kuikman, 1990; Bethlenfalvay and Lindermann, 1992; Daily, 1995; Arshad *et al.*, 1996; Coleman, 1996; Jastrow and Miller, 1997; Young *et al.*, 1998).”

In addition, Gonzalez-Chavez *et al.* (2004) found “glomalin participates in the sequestration of different PTEs [potentially toxic elements],” “the glomalin pool in the soil may have a potential to sequester PTEs, not only by the colonized roots, but also by the hyphae and through deposition of glomalin in soil,” and “this glycoprotein may be stabilizing PTEs, reducing PTE availability and decreasing the toxicity risk to other soil microorganisms and plants.” The authors note “glomalin is ... copiously produced by all AMF tested to date (Wright *et al.*, 1996, 1998; Nichols, 2003),” “AMF colonize 80% of vascular plant species (Trappe, 1987),” and AMF “are found worldwide in almost every soil.”

In light of these observations, it should be evident the ongoing rise in the air’s CO<sub>2</sub> content must be having a tremendous positive impact on the biosphere via a suite of mechanisms linked to a fungal-produced protein that only a couple decades ago was largely unknown—even to most plant and soil scientists—and similarly unappreciated.

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## 2.9 Isoprene

A number of experimental findings indicate the ongoing rise in the air's CO<sub>2</sub> content likely will lead to significant reductions in the air's concentration of isoprene, which is responsible for the production of vast amounts of tropospheric ozone, thereby helping to reduce the atmospheric presence of the latter noxious trace gas. Other research suggests a CO<sub>2</sub>-induced reduction in plant isoprene emissions will result in greater concentrations of cloud condensation nuclei, which act as a negative feedback to atmospheric warming.

The influence of atmospheric CO<sub>2</sub> on plant isoprene emissions is discussed in Section 2.5.2.2.1 of the Forcings and Feedbacks chapter of *Climate Change Reconsidered II: Physical Science* (Idso *et al.*, 2013).

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## 2.10 Leaves

Nearly all crops respond to increases in the air's CO<sub>2</sub> content by displaying enhanced rates of photosynthesis and biomass production. The subsections below examine some of the other changes they often experience when exposed to elevated levels of atmospheric CO<sub>2</sub>—namely, various changes in leaf characteristics.

### 2.10.1 Agricultural Species

- Increasing atmospheric CO<sub>2</sub> concentrations likely will affect many leaf characteristics of agricultural plants, with the majority of the changes leading to higher rates and efficiencies of photosynthesis and growth, with the ultimate result that agricultural yields likely will rise as the air's CO<sub>2</sub> content continues to rise.

As the air's CO<sub>2</sub> concentration rises, plants commonly reduce the concentration of the nitrogen-rich photosynthetic enzyme rubisco, which is normally present in their leaves in excess amounts at ambient atmospheric CO<sub>2</sub> concentrations. Consequently, elevated CO<sub>2</sub> exposure frequently results in reduced foliar nitrogen concentrations, which allows excess nitrogen in leaves to be mobilized away from the photosynthetic apparatus and devoted to processes more limiting to growth. In the study of Monje and Bugbee (1998), for example, a near-900-ppm increase in the air's CO<sub>2</sub> concentration reduced leaf nitrogen contents in wheat by 28%. Similar results were obtained for soybeans grown at twice-ambient CO<sub>2</sub> concentrations (Sims *et al.*, 1998); it is thus quite likely many agricultural species will exhibit reductions in foliar nitrogen content in response to atmospheric CO<sub>2</sub> enrichment.

In addition to nitrogen, elevated CO<sub>2</sub> also can mobilize other limiting resources away from a plant's photosynthetic machinery and direct them into various plant parts important to growth and development. Watling *et al.* (2000), for example, found a doubling of the air's CO<sub>2</sub> content reduced the thickness of specialized bundle sheath cells in sorghum by approximately 50%, which freed up important resources that were sent to other parts of the plant for utilization, as indicated by a 36% enhancement in total plant biomass.

Elevated CO<sub>2</sub> also may impact leaf concentrations of chlorophylls, important light-absorbing pigments involved in the photosynthetic process. In the study of Sgherri *et al.* (1998), for example, water-stressed

alfalfa displayed a 30% reduction in leaf chlorophyll content, but water-stressed plants exposed to 600 ppm CO<sub>2</sub> exhibited only a 6% reduction. In potato, on the other hand, Sicher and Bunce (1999) found no change in leaf chlorophyll content when exposing plants to twice-ambient levels of atmospheric CO<sub>2</sub>, nor did Monje and Bugbee (1998) in their previously mentioned study on wheat.

Excess carbohydrates resulting from enhanced photosynthetic rates often are used to increase leaf growth and development. As an example, Reddy *et al.* (1998) reported cotton plants grown at 700 ppm CO<sub>2</sub> displayed individual leaf areas 20% greater than those of the leaves of ambiently grown control plants. Similarly, Masle (2000) noted a 600-ppm increase in atmospheric CO<sub>2</sub> concentration increased individual leaf size in wheat plants by increasing the number of photosynthetic mesophyll cell layers, as well as overall leaf thickness. In a related study on sunflower, however, Sims *et al.* (1999) did not report an increase in leaf size or thickness, but they documented a CO<sub>2</sub>-induced shift in the distribution of leaf area that concentrated 30 to 40% more of it in the upper layer of the plant canopy, where the CO<sub>2</sub>-induced photosynthetic stimulation was greatest.

Sometimes, excess carbohydrates are used to enhance the biosynthesis of secondary carbon compounds in leaves. Estiarte *et al.* (1999), for example, found leaves of spring wheat grown at 550 ppm CO<sub>2</sub> displayed 14% higher total flavonoid concentrations than leaves of plants grown at 370 ppm CO<sub>2</sub>. This observation is important because flavonoids are generally characterized as having anti-herbivory properties. Thus, less pest-induced yield losses in this important grain crop may occur in future climates characterized by elevated atmospheric CO<sub>2</sub> concentrations.

Increasing atmospheric CO<sub>2</sub> concentrations likely will affect many leaf characteristics of agricultural plants. The majority of these changes likely will lead to greater rates and higher efficiencies of photosynthesis and growth. As a result, agricultural yields likely will rise in the future, resulting in part from changes in foliar properties mediated by the ongoing rise in the air's CO<sub>2</sub> content.

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### 2.10.2 Trees

- The leaves of nearly all woody species exposed to increases in the air's CO<sub>2</sub> content display enhanced rates of photosynthesis, as well as a number of other changes in leaf characteristics that help promote increased resistance to attacks by herbivores and pathogens.

Norby *et al.* (2000) found a 300-ppm increase in the air's CO<sub>2</sub> concentration reduced the nitrogen content in the leaves of red and sugar maple trees by 19 and 25%, respectively, and Rey and Jarvis (1998) found young silver birch trees exposed to twice-ambient levels of atmospheric CO<sub>2</sub> displayed leaf nitrogen contents 13% below those observed in leaves of ambiently growing trees. This phenomenon also has

been noticed in the needles of conifers. Gielen *et al.* (2000), for example, documented a 33% reduction in the needle nitrogen content of young Scots pines growing at 750 ppm CO<sub>2</sub> compared to that of control trees growing at 350 ppm CO<sub>2</sub>.

The nitrogen thus given up by the photosynthetic machinery of both broad-leaved and coniferous trees typically is used to further enhance the overall biomass production of the trees, including roots, trunks, branches, and still more leaves.

Elevated CO<sub>2</sub> also can mobilize other limiting resources away from the photosynthetic process for input into still other processes important to tree growth and development. Ormrod *et al.* (1999), for example, found a 180-ppm increase in the air's CO<sub>2</sub> content caused 19 and 25% reductions in the chlorophyll *a* and *b* concentrations of Douglas fir needles, respectively. Similar results were reported in young Scots pines, which exhibited a 26% reduction in total needle chlorophyll concentration in response to a 350-ppm increase in the CO<sub>2</sub> content of the air (Gielen *et al.*, 2000). However, Carter *et al.* (2000) noted elevated CO<sub>2</sub> (+300 ppm) had no effect on leaf chlorophyll concentrations in sugar maple.

Excess carbohydrates resulting from CO<sub>2</sub>-enhanced photosynthesis often are used close to their site of production to increase leaf growth. In reviewing the results of several peer-reviewed papers related to this topic, Taylor *et al.* (2001) conclude elevated CO<sub>2</sub> consistently enhanced leaf extension rates in poplar species, which positively correlates with increased wood production. Similarly, Ferris *et al.* (2001) report a 200-ppm increase in the air's CO<sub>2</sub> concentration boosted leaf area in three poplar species by approximately 40%. In addition, a doubling of the atmospheric CO<sub>2</sub> concentration enhanced leaf size in a native British tree species (*Alnus glutinosa*) by 17% (Poole *et al.*, 2000). In all of these cases, it is likely CO<sub>2</sub>-induced increases in leaf turgor pressure (Tognetti *et al.*, 2000) contributed to the CO<sub>2</sub>-induced enhancements in leaf growth, as mediated through enhanced cell division and elongation.

Sometimes, excess carbohydrates are used to enhance the biosynthesis of secondary carbon compounds within leaves, which often results in greater specific leaf areas (Cornelissen *et al.*, 1999) and defensive resistance to pathogens and herbivores. Hattenschwiler *et al.* (1999) found a 280-ppm increase in the air's CO<sub>2</sub> concentration significantly increased needle concentrations of tannins and phenolics in spruce trees. Similarly, a 350-ppm increase in the CO<sub>2</sub> content of the air enhanced total phenolics in needles of loblolly pine by 21%

(Gebauer *et al.*, 1998). Leaves of various Mediterranean forest species grown at 710 ppm CO<sub>2</sub> displayed 18% greater lignin concentrations than leaves from species grown at 350 ppm CO<sub>2</sub> (De Angelis *et al.*, 2000). However, Heyworth *et al.* (1998) did not observe any significant effects of elevated CO<sub>2</sub> on tannin concentrations in needles of Scots pine exposed to 700 ppm CO<sub>2</sub>, nor did King *et al.* (2001) for aspen leaves exposed to the same atmospheric CO<sub>2</sub> concentration. And Schaffer *et al.* (1997) found twice-ambient levels of atmospheric CO<sub>2</sub> stimulated the production of foliar carbon compounds in mango trees so dramatically that the concentrations of several leaf minerals fell due to this “dilution effect.”

Under certain conditions, excess carbohydrates also are used to enhance various leaf anatomical features. Paoletti *et al.* (1998), for example, report white oak trees fumigated with air containing 750 ppm CO<sub>2</sub> displayed leaf cuticles three times thicker than those of leaves on trees grown in air containing 350 ppm CO<sub>2</sub>. Lin *et al.* (2001) report this same elevated CO<sub>2</sub> concentration enhanced needle thickness in young Scots pines by increasing the area occupied by photosynthetic mesophyll tissue. Such increases in leaf and cuticle thickness can increase resistance to herbivory and pathogenic attack, much as increased concentrations of secondary carbon compounds do.

It would appear the current upward trend in atmospheric CO<sub>2</sub> concentration will affect numerous leaf characteristics of woody plants. The available data suggest the resulting changes likely will lead to greater and more efficient photosynthesis and growth rates, together with increased leaf resistance to herbivory and pathogen attack. Thus, tree productivity likely will continue to increase in the future due to changes in foliar properties driven by the ongoing rise in the air's CO<sub>2</sub> content.

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### 2.10.3 Other

- The ongoing rise in the air's CO<sub>2</sub> content will alter many physical and physiological leaf characteristics of Earth's plants; some of these changes will lead to more efficient plant growth, including the production of important secondary carbon compounds possessing high potential for combating a number of human maladies.

Nearly all plants respond to increases in the air's CO<sub>2</sub> content by displaying enhanced rates of photosynthesis and biomass production. Simultaneously, plants often exhibit changes in leaf characteristics. This review summarizes some of the CO<sub>2</sub>-induced leaf responses observed in non-agricultural and non-woody species.

When the air's CO<sub>2</sub> concentration is experimentally increased, plants commonly reduce their foliar concentrations of the nitrogen-rich photosynthetic enzyme rubisco. Consequently, exposure to elevated CO<sub>2</sub> concentrations frequently results in lowered foliar nitrogen concentrations. This adjustment allows excess nitrogen to be mobilized away from the photosynthetic process and into processes more limiting to growth.

Schappi and Korner (1997), for example, note two species common to the Swiss Alps displayed reduced foliar nitrogen concentrations in response to

atmospheric CO<sub>2</sub> enrichment. However, Goverde *et al.* (1999) found no change in the leaf nitrogen contents of *Lotus corniculatus* plants exposed to twice-ambient atmospheric CO<sub>2</sub> concentrations, nor did David *et al.* (2001) in leaves of *Medicago* and *Trifolium* species. In the latter study, the authors report a CO<sub>2</sub>-induced *increase* in leaf nitrogen content for a certain *Galactites* species. Nevertheless, in a review of 67 published experimental observations made on several dozen species, Norby *et al.* (2001) conclude a doubling of the air's CO<sub>2</sub> concentration reduced leaf nitrogen content by, on average, approximately 7%.

At the other end of the spectrum of leaf responses to atmospheric CO<sub>2</sub> enrichment, excess carbohydrates resulting from CO<sub>2</sub>-induced increases in photosynthetic rates often are used to enhance the biosynthesis of secondary carbon compounds. Norby *et al.* (2001), for example, analyzed 46 published experimental observations and determined a doubling of the atmospheric CO<sub>2</sub> content increased leaf lignin concentrations in a number of species by an average of 6.5%. Leaf chlorophyll contents also have been reported to increase in young orchid plantlets exposed to elevated CO<sub>2</sub> concentrations (Gouk *et al.*, 1999). Also, Goverde *et al.* (1999) observed elevated CO<sub>2</sub> concentrations increased leaf tannin concentrations in *Lotus corniculatus*, although Kerslake *et al.* (1998) could not discern any CO<sub>2</sub> effect on foliar phenolic concentrations in *Calluna vulgaris*.

Idso and Idso (2001) note a near-tripling of the air's CO<sub>2</sub> content enhanced the concentration of the heart-helping compound digoxin in the woolly foxglove by about 12%, and a 75% increase in the air's CO<sub>2</sub> content produced 6 to 28% increases in the concentrations of five substances produced by spider lilies that have proven effective in treating a number of human cancers and viral diseases.

The ongoing rise in the air's CO<sub>2</sub> concentration will alter many physical and physiological leaf characteristics of Earth's plants. The data suggest the resulting changes will lead to more efficient plant growth and increase the production of secondary carbon compounds, some of which have high potential for combating human diseases. Thus, the increasing CO<sub>2</sub> content of the air may indirectly—but significantly—improve the quality of human life.

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## 2.11 Lignin

- Atmospheric CO<sub>2</sub> enrichment stimulates photosynthesis in nearly all plants, enabling them to produce more nonstructural carbohydrates that can be used to create important carbon-based secondary compounds, one of which is lignin.

Lignin is important because it tends to inhibit the biodegradation of organic materials. Some studies suggest plant lignin concentrations tend to decline in response to increases in the air's CO<sub>2</sub> concentration. In one such study—a five-year open-top chamber experiment—Cotrufo and Ineson (2000) found a doubling of the air's CO<sub>2</sub> concentration decreased lignin concentrations in twigs of beech seedlings by approximately 12%. Nevertheless, after 42 months of incubation in native forest soils, the decomposition rate of the CO<sub>2</sub>-enriched twigs was still 5% less than that of the twigs grown in ambient air. Likewise, in an open-top chamber study of an agricultural crop (tall fescue), Newman *et al.* (2003) found doubling the air's CO<sub>2</sub> content reduced forage lignin concentration

by 14%.

At the other end of the spectrum, a larger number of experiments has revealed CO<sub>2</sub>-induced increases in plant lignin concentrations. With the CO<sub>2</sub>-induced production of more lignin in plant tissues, the ongoing rise in the air's CO<sub>2</sub> content could lead to enhanced carbon sequestration in the world's soils, because plant-produced organic matter supplied to soils would be more resistant to decomposition; the end result of this phenomenon would be a slowing of the rate-of-rise of the atmosphere's CO<sub>2</sub> concentration, which could reduce the magnitude of CO<sub>2</sub>-induced global warming.

In a two-year study of ash and sycamore seedlings grown in closed-top chambers (solar domes) at CO<sub>2</sub> concentrations of either 350 ppm (ambient) or 600 ppm (enriched), Cotrufo *et al.* (1998) observed greater concentrations of lignin in the litter produced by both species, and after one year of incubating the litter from the two CO<sub>2</sub> treatments in bags placed within a forest soil, the bags containing the litter produced by the CO<sub>2</sub>-enriched trees of both species had about 30% more dry mass remaining in them than did the bags containing the litter produced by the ambient-treatment trees. In addition, woodlouse arthropods consumed 16% less litter in the CO<sub>2</sub>-enriched chambers than in the ambient-treatment chambers.

Higher plant lignin concentrations also were observed in oak seedlings growing in doubled-CO<sub>2</sub> air (700 ppm as opposed to 350 ppm) in the controlled-environment greenhouse study of Staudt *et al.* (2001), as well as in trembling aspen seedlings growing in doubled-CO<sub>2</sub> air in the open-top chamber study of Tuchman *et al.* (2003). And in an open-top chamber study of a 30-year-old mixed-stand of various Mediterranean forest species growing near the coast of central Italy, De Angelis *et al.* (2000) determined doubled-CO<sub>2</sub> air increased leaf-litter lignin concentrations by 18%, which was accompanied by an 8% reduction in the initial loss of mass from the decomposing CO<sub>2</sub>-enriched litter and a 4% reduction one year later.

Booker *et al.* (2000) observed higher lignin concentrations in the roots of CO<sub>2</sub>-enriched cotton, a perennial woody plant, as did Booker *et al.* (2005) in CO<sub>2</sub>-enriched soybean, an annual herbaceous plant. They conclude, “one result of increased residue production and higher levels of recalcitrant material such as lignin being added to the soil is that soil carbon sequestration should increase, a response anticipated to occur with increasing concentrations of atmospheric CO<sub>2</sub>.”

More often than not, however, experiments of this type have found no significant changes in the tissue lignin concentrations of plants grown in CO<sub>2</sub>-enriched air. Studies in this category include the work of Hirschel *et al.* (1997) dealing with plants growing in an alpine grassland, a lowland calcareous grassland, and a lowland, wet, tropical rainforest; Booker and Maier (2001) and Finzi and Schlesinger (2002) dealing with loblolly pine trees; Peñuelas *et al.* (2002) investigating three species of shrub in Pisa, Italy; and Billings *et al.* (2003) dealing with four species of shrub in the Mojave Desert, Nevada, USA.

Norby *et al.* (2001) conducted a meta-analytic review that considered this issue from a number of different perspectives. Based on 46 experimental observations, they determined elevated atmospheric CO<sub>2</sub> concentrations increased leaf-litter lignin concentrations by an average of 6.5%. These increases in lignin content occurred only in woody species. Leaf-litter lignin concentrations were not affected by elevated CO<sub>2</sub> when plants were grown in open-top chambers, FACE plots, or in the proximity of CO<sub>2</sub>-emitting springs. In analyzing 101 observations, Norby *et al.* found elevated CO<sub>2</sub> had no consistent effect on leaf litter decomposition rate in any experimental setting.

Almost all of the studies conducted to date on woody species, where there is some evidence of an increase in leaf-litter lignin concentration (and there thus remains a possibility of CO<sub>2</sub>-enhanced carbon sequestration), have been of short duration compared to the lifespans of various forest components. The long-term consequences of modest CO<sub>2</sub>-induced increases in woody-plant lignin concentrations for soil carbon sequestration will not be known until several experiments of much longer duration than any conducted so far have been conducted. Nevertheless, as Booker *et al.* (2005) remind us, because there is also considerably more litter produced by almost all plants in CO<sub>2</sub>-enriched air, soil carbon sequestration should continue to increase as the air's CO<sub>2</sub> content continues to rise.

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## 2.12 Lipids

- The highly positive effects of atmospheric CO<sub>2</sub> enrichment on lipid concentrations and characteristics in various terrestrial and aquatic plants portend benefits for both the managed and unmanaged components of Earth's biosphere as the air's CO<sub>2</sub> content continues to climb.

Lipids are hydrophobic molecules consisting mainly of fats, oils, and waxes that possess long non-polar hydrocarbon groups arranged in chains commonly called fatty acid chains, one type of which (phospholipids) is a major component of plant and animal membranes. They are extremely important to the well-being of nearly all living organisms.

Seeking to discover how lipids are affected by elevated concentrations of atmospheric CO<sub>2</sub>, Sgherri *et al.* (1998) grew alfalfa in open-top chambers at ambient (340 ppm) and enriched (600 ppm) CO<sub>2</sub> concentrations for 25 days. They then withheld water from the plants for five additional days. They found the plants grown at the elevated CO<sub>2</sub> concentration maintained greater leaf lipid to protein ratios, especially under conditions of water stress. In addition, leaf lipid contents of the plants grown in the CO<sub>2</sub>-enriched air were 22 and 83% greater than those of the plants grown in ambient air for periods of ample and insufficient soil moisture, respectively. Also, the degrees of unsaturation of two of the most important lipids of the thylakoid membranes (which are found within chloroplasts and contain chlorophyll, other pigments that absorb sunlight, and specialized protein complexes that convert sunlight into usable cellular energy during photosynthesis) were approximately 20 and 37% greater in the high-compared to the low-CO<sub>2</sub> treatments during times of adequate and inadequate soil moisture, respectively.

Sgherri *et al.* suggest the greater lipid contents observed in CO<sub>2</sub>-enriched air and their increased degree of unsaturation may allow thylakoid membranes to maintain a more fluid and stable environment, critical during periods of water stress in enabling plants to continue photosynthetic carbon uptake. Some researchers have suggested adaptive plant responses such as these may allow plants to better cope with *any* altered environmental condition that produces stress.

With greater amounts of CO<sub>2</sub> in the atmosphere, the resulting greater lipid contents of thylakoid membranes plus their greater degree of unsaturation may provide greater membrane stability and integrity, thereby allowing proper functioning in times of drought or other adverse environmental conditions. In

some cases, these CO<sub>2</sub>-induced adaptations may mean the difference between a plant's living or dying.

Consider, for example, the stress of exposure to overly cold temperatures that often leads to chilling injury in plants and the role played by membrane lipid unsaturation in alleviating those injuries.

Hugly and Somerville (1992) worked with wild-type *Arabidopsis thaliana* and two mutants of the species that were deficient in thylakoid lipid unsaturation. They found "chloroplast membrane lipid polyunsaturation contributes to the low-temperature fitness of the organism," and that it is required for some aspect of chloroplast biogenesis. When lipid polyunsaturation was low, for example, they observed what they called "dramatic reductions in chloroplast size, membrane content, and organization in developing leaves." They found a positive correlation "between the severity of chlorosis in the two mutants at low temperatures and the degree of reduction in polyunsaturated chloroplast lipid composition."

Kodama *et al.* (1994) demonstrate the low-temperature-induced suppression of leaf growth and concomitant induction of chlorosis observed in wild-type plants is much less evident in transgenic plants containing a gene that allows for greater expression of unsaturation in the fatty acids of leaf lipids. They conclude substantially unsaturated fatty acids "are undoubtedly an important factor contributing to cold tolerance."

Moon *et al.* (1995) found heightened unsaturation of the membrane lipids of chloroplasts stabilized the photosynthetic machinery of transgenic tobacco plants against low-temperature photo-inhibition "by accelerating the recovery of the photosystem II protein complex." Kodama *et al.* (1995), also working with transgenic tobacco plants, showed increased fatty acid desaturation is one of the prerequisites for normal leaf development at low, nonfreezing temperatures. Ishizaki-Nishizawa *et al.* (1996) found transgenic tobacco plants with a reduced level of saturated fatty acids in most membrane lipids "exhibited a significant increase in chilling resistance."

These observations are laden with significance for Earth's agro-ecosystems, since many economically important crops, such as rice, maize, and soybeans, are classified as chilling-sensitive: they experience injury or death at temperatures between 0 and 15°C (Lyons, 1973). If atmospheric CO<sub>2</sub> enrichment enhances their production of thylakoid lipids and their degree of unsaturation, as it does in alfalfa, a continuation of the ongoing rise in the air's CO<sub>2</sub>

content could increase the abilities of these agricultural species to withstand periodic exposure to debilitating low temperatures; this phenomenon could provide the extra boost to food production that will be needed to sustain the planet's increasing numbers in the future (Tilman *et al.*, 2001).

Hussain *et al.* (2001) collected and analyzed seeds from loblolly pine trees exposed to atmospheric CO<sub>2</sub> concentrations of either 350 or 560 ppm since 1996 in the Duke Forest FACE study. The seeds collected from the CO<sub>2</sub>-enriched trees were 90% heavier than seeds collected from the trees growing in ambient air, and their mean lipid content was 265% greater. The germination success of seeds developed under CO<sub>2</sub>-enriched conditions was more than three times greater than that of the seeds from the ambient-air treatment, regardless of germination CO<sub>2</sub> concentration. In addition, seeds from the CO<sub>2</sub>-enriched trees germinated approximately five days earlier, regardless of germination CO<sub>2</sub> concentration, and the seedlings derived from the seeds collected from the CO<sub>2</sub>-enriched trees displayed significantly greater root lengths and needle numbers than those derived from seeds collected from the ambient-treatment trees.

Schwanz and Polle (1998) evaluated the degree of lipid peroxidation in leaves of mature holm and white oak trees that had been growing in the vicinity of natural CO<sub>2</sub> springs in central Italy for close to half a century. They found the trees growing in close proximity to the CO<sub>2</sub>-emitting springs often exhibited lipid peroxidation reductions, which indicate less intrinsic oxidative stress and the presence of fewer internal harmful oxidants.

Yu *et al.* (2004) grew the marine microalgae *Platymonas subcordiformis* in the laboratory at ambient levels of atmospheric CO<sub>2</sub> concentration and UV-B radiation flux density, as well as at elevated levels of 5,000 ppm CO<sub>2</sub> and the UV-B radiation flux anticipated to result from a 25% stratospheric ozone depletion under clear sky conditions in summer. They report the elevated UV-B treatment by itself significantly increased the production of the toxic superoxide anion and hydrogen peroxide, as well as malonyldialdehyde, an end product of lipid peroxidation. Elevated CO<sub>2</sub> by itself did just the opposite. In the treatment consisting of elevated UV-B and elevated CO<sub>2</sub>, the concentrations of these three substances were all lower than those observed in the elevated UV-B and ambient CO<sub>2</sub> treatment. Yu *et al.* conclude, "CO<sub>2</sub> enrichment could reduce oxidative stress of reactive oxygen species to *P. subcordiformis*, and reduce the lipid peroxidation damage of UV-B to

*P. subcordiformis*."

An interesting aspect of these findings is what they imply about coral bleaching. In the introduction to their review of this important subject, Smith *et al.* (2005) report, "photoinhibition of photosynthesis and photodamage to photosystem II of the zooxanthellae, with the consequent increase in the production of damaging reactive oxygen species (ROS), have been implicated as the cause of thermal bleaching (Brown, 1997; Fitt *et al.*, 2001; Lesser, 2004; Tchernov *et al.*, 2004)." At the end of their review, they report the "thermal bleaching of many corals is ultimately the result of the destruction of photosynthetic pigments by ROS," and the production by the zooxanthellae of one particular ROS, hydrogen peroxide, "may be a signal that triggers a response in the host cell to eject the zooxanthellae or shed the host cell from the coral." Combining these observations with the finding of Yu *et al.* that CO<sub>2</sub> enrichment counters the production of hydrogen peroxide, it follows that some degree of atmospheric CO<sub>2</sub> enrichment should likewise cause host cells to not eject their zooxanthellae.

Goverde *et al.*, 2002) examined the impact of atmospheric CO<sub>2</sub> enrichment on lipid concentrations in the body of an animal, specifically, the satyrid butterfly (*Coenonympha pamphilus*), larvae of which were raised in semi-natural, undisturbed calcareous grassland plots exposed to atmospheric CO<sub>2</sub> concentrations of 370 and 600 ppm for five growing seasons. Among other things, this work revealed the elevated atmospheric CO<sub>2</sub> concentration increased lipid concentrations in the bodies of adult male butterflies by nearly 14%. Since these compounds are used as energy resources in these and other butterflies, this animal species—and perhaps others—likely will exhibit positive responses to future increases in the air's CO<sub>2</sub> concentration.

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## 2.13 Low Temperature

- The stresses of low temperature have been shown to be both alleviated and enhanced in plants growing under elevated CO<sub>2</sub> concentrations. It appears far more plants are benefiting from such stress alleviation than are experiencing stress augmentation.

Loik *et al.* (2000) grew three *Yucca* species (*brevifolia*, *schidigera*, and *whipplei*) in pots placed in glasshouses maintained at atmospheric CO<sub>2</sub> concentrations of 360 and 700 ppm and day/night air temperatures of 40/24°C for seven months, after which some of the plants were subjected to a two-week day/night air temperature treatment of 20/5°C. In addition, leaves from each *Yucca* species were removed and placed in a freezer cooled at a rate of 3°C per hour until a minimum temperature of -15°C was reached. These manipulations indicated elevated CO<sub>2</sub> lowered the air temperature at which 50% low-temperature-induced cell mortality occurred by 1.6, 1.4, and 0.8°C in *brevifolia*, *schidigera*, and *whipplei*, respectively. On the basis of the result obtained for *Y. brevifolia*, Dole *et al.* (2003) estimate “the increase in freezing tolerance caused by doubled CO<sub>2</sub> would increase the potential habitat of this species by 14%.”

Obrist *et al.* (2001) observed just the opposite response. In an open-top chamber study of a temperate grass ecosystem growing on a nutrient-poor calcareous soil in northwest Switzerland, portions of which had been exposed to atmospheric CO<sub>2</sub> concentrations of 360 and 600 ppm for six years, they found the average temperature at which 50% low-temperature-induced leaf mortality occurred in five prominent species rose by an average of 0.7°C in response to the extra 240 ppm of CO<sub>2</sub> employed in their experiment.

Most relevant investigations, however, have

produced evidence of positive CO<sub>2</sub> effects on plants' low-temperature tolerance. Sigurdsson (2001), for example, grew black cottonwood seedlings near Gunnarsholt, Iceland within closed-top chambers maintained at ambient and twice-ambient atmospheric CO<sub>2</sub> concentrations for three years, finding elevated CO<sub>2</sub> tended to hasten the end of the growing season. This effect was interpreted as enabling the seedlings to better avoid the severe cold-induced dieback of newly produced tissues that often occurs with the approach of winter in this region. Likewise, Wayne *et al.* (1998) found yellow birch seedlings grown at an atmospheric CO<sub>2</sub> concentration of 800 ppm exhibited greater dormant bud survivorship at low air temperatures than did seedlings grown at 400 ppm CO<sub>2</sub>.

Schwanz and Polle (2001) investigated the effects of elevated CO<sub>2</sub> on chilling stress in micropropagated hybrid poplar clones that were subsequently potted and transferred to growth chambers maintained at either ambient (360 ppm) or elevated (700 ppm) CO<sub>2</sub> for three months. They found "photosynthesis was less diminished and electrolyte leakage was lower in stressed leaves from poplar trees grown under elevated CO<sub>2</sub> as compared with those from ambient CO<sub>2</sub>." Although severe chilling caused pigment and protein degradation in all stressed leaves, the damage was expressed to a lower extent in leaves from the elevated CO<sub>2</sub> treatment. This CO<sub>2</sub>-induced chilling protection was accompanied by a rapid induction of superoxide dismutase activity and by slightly higher stabilities of other antioxidative enzymes.

Sgherri *et al.* (1998) report raising the air's CO<sub>2</sub> concentration from 340 to 600 ppm increased lipid concentrations in alfalfa thylakoid membranes while inducing a higher degree of unsaturation in the most prominent of those lipids. Under well-watered conditions, for example, the 76% increase in atmospheric CO<sub>2</sub> enhanced overall thylakoid lipid concentration by about 25%, while it increased the degree of unsaturation of the two main lipids by approximately 17% and 24%. Under conditions of water stress, these responses were even greater, as thylakoid lipid concentration rose by approximately 92%, and the degree of unsaturation of the two main lipids rose by about 22% and 53%.

What these observations have to do with a plant's susceptibility to chilling injury is evident from a number of studies conducted over the past decade. For example, Hugly and Somerville (1992) studied wild-type *Arabidopsis thaliana* and two mutants deficient in thylakoid lipid unsaturation, finding "chloroplast membrane lipid polyunsaturation

contributes to the low-temperature fitness of the organism" and it "is required for some aspect of chloroplast biogenesis." When lipid polyunsaturation was low, they observed "dramatic reductions in chloroplast size, membrane content, and organization in developing leaves." They found a positive correlation "between the severity of chlorosis in the two mutants at low temperatures and the degree of reduction in polyunsaturated chloroplast lipid composition."

Kodama *et al.* (1994) demonstrate the low-temperature-induced suppression of leaf growth and concomitant induction of chlorosis observed in wild-type tobacco plants is much less evident in transgenic plants containing a gene that allows for greater expression of unsaturation in the fatty acids of leaf lipids. They conclude substantially unsaturated fatty acids "are undoubtedly an important factor contributing to cold tolerance."

Moon *et al.* (1995) found heightened unsaturation of the membrane lipids of chloroplasts stabilized the photosynthetic machinery of transgenic tobacco plants against low-temperature photoinhibition "by accelerating the recovery of the photosystem II protein complex." Likewise, Kodama *et al.* (1995), also working with transgenic tobacco plants, showed increased fatty acid desaturation is one of the prerequisites for normal leaf development at low, nonfreezing temperatures. Ishizaki-Nishizawa *et al.* (1996) demonstrate transgenic tobacco plants with a reduced level of saturated fatty acids in most membrane lipids "exhibited a significant increase in chilling resistance."

Many economically important crops, such as rice, maize, and soybeans, are classified as chilling-sensitive—they experience injury or death at temperatures between 0 and 15°C (Lyons, 1973). If atmospheric CO<sub>2</sub> enrichment enhances their production and degree-of-unsaturation of thylakoid lipids, as it does in alfalfa, a continuation of the ongoing rise in the air's CO<sub>2</sub> content could increase the abilities of these critically important agricultural species to withstand periodic exposure to debilitating low temperatures, and this could provide the extra boost in food production that will be needed to sustain the planet's increasing numbers in the years and decades ahead.

Earth's natural ecosystems also would benefit from a CO<sub>2</sub>-induced increase in thylakoid lipids containing more-highly-unsaturated fatty acids. Many plants of tropical origin, for example, suffer cold damage when temperatures fall below 20°C (Graham and Patterson, 1982). With improved lipid charac-

teristics provided by the ongoing rise in the air's CO<sub>2</sub> content, such plants would be able to expand their ranges both poleward and upward in a higher-CO<sub>2</sub> world.

Baczek-Kwinta and Koscielniak (2003) grew two hybrid maize (*Zea mays* L.) genotypes—KOC 9431 (chill-resistant) and K103xK85 (chill-sensitive)—from seed in air of either ambient (350 ppm) or elevated (700 ppm) CO<sub>2</sub> concentration (AC or EC, respectively), after which the plants were exposed to air of 7°C for 11 days and then recovered in ambient air of 20°C for one day. Throughout this period, physiological and biochemical parameters were measured on the plants' third fully expanded leaves.

Under such conditions, Baczek-Kwinta and Koscielniak report, "EC inhibited chill-induced depression of net photosynthetic rate (PN), especially in leaves of chill-resistant genotype KOC 9431." This phenomenon "was distinct not only during chilling, but also during the recovery of plants at 20°C." They note, "seedlings subjected to EC showed 4-fold higher PN when compared to AC plants." They also determined "EC diminished the rate of superoxide radical formation in leaves in comparison to the AC control." In addition, "electrolyte leakage from the [leaf membrane] tissue, a parameter reflecting membrane injury, was significantly lower in samples of plants subjected to EC than AC." Finally, they report enrichment of air with CO<sub>2</sub> successfully inhibited the decrease in the maximal quantum efficiency of photosystem 2, both after chilling and during the one-day recovery period. Taking all these positive effects of elevated CO<sub>2</sub> together, the authors conclude, "the increase in atmospheric CO<sub>2</sub> concentration seems to be one of the protective factors for maize grown in cold temperate regions."

Melkonian *et al.* (2004) grew well-watered and fertilized mature bean plants (*Phaseolus vulgaris* L., cv. Bush Blue Lake 274) in CO<sub>2</sub>-controlled growth chambers maintained at concentrations of either 350 or 700 ppm at 25/18°C day/night temperatures. The plants then received 24 hours of chilling at 6.5°C (8 hours light, 10 hours dark, 6 hours light), after which there was a 24-hour recovery period under pre-chill growing conditions, followed by a second 24 hours of chilling and a 48-hour recovery period consisting of pre-chill conditions. Throughout this time, net photosynthesis measurements were made two to three hours before and after each shift in temperature.

According to the authors, prior to chilling, net photosynthesis was approximately 20% higher at elevated compared to ambient CO<sub>2</sub>. During chilling, plants of both treatments exhibited near-identical

much-reduced values. Subsequently, however, and "consistent with previous research [Boese *et al.*, 1997] on this cultivar," in the words of Melkonian *et al.*, "post-chilling recovery was more rapid at elevated compared to ambient CO<sub>2</sub>."

Alam *et al.* (2005) grew eight-month-old saplings of two clones (PB 235 and RRII 105) of *Hevea brasiliensis*, a tree species indigenous to humid tropical climates, in 0.75-m<sup>3</sup> polybags filled with garden soil during the two coldest months of the year in warm and cool regions of peninsular India having mean minimum temperatures of 22.0 and 10.0°C, respectively, while they measured a number of photosynthetic properties of the trees' leaves. The authors report, "irrespective of the differences in growth environment, higher CO<sub>2</sub> in the ambient air during measurements improved the photochemical efficiency of the plants as reflected in higher net photosynthetic rate, effective photosystem 2 quantum efficiency, and photochemical quenching." The Indian scientists conclude their results imply "inhibition in photochemical efficiency due to sub-optimal temperatures could be improved considerably with higher CO<sub>2</sub> concentration by making more CO<sub>2</sub> available to photosynthesis."

Kostiainen *et al.* (2006) studied the effects of elevated carbon dioxide (CO<sub>2</sub>) and ozone (O<sub>3</sub>) on various wood properties of two initially seven-year-old fast-growing silver birch (*Betula pendula* Roth) clones grown out-of-doors at Suonenjoki, Finland for three additional years in open-top chambers maintained at ambient and 1.9x ambient CO<sub>2</sub> concentrations in combination with ambient and 1.5x ambient O<sub>3</sub> concentrations. The Finnish researchers note "the concentration of nonstructural carbohydrates (starch and soluble sugars) in tree tissues is considered a measure of carbon shortage or surplus for growth (Korner, 2003)." Hence, they say "starch accumulation observed under elevated CO<sub>2</sub> in this study indicates a surplus of carbohydrates produced by enhanced photosynthesis of the same trees (Riikonen *et al.*, 2004)." In addition, they report, "during winter, starch reserves in the stem are gradually transformed to soluble carbohydrates involved in freezing tolerance (Bertrand *et al.*, 1999; Piispanen and Saranpaa, 2001), so the increase in starch concentration may improve acclimation in winter."

Bigras and Bertrand (2006) grew well-watered and fertilized black spruce (*Picea mariana* Mill. BSP) plants from seed in containers filled with a 3:1 mixture of peat:vermiculite in mini-greenhouses located within a larger greenhouse where they were

exposed—at ambient and elevated (370 and 710 ppm) concentrations of atmospheric CO<sub>2</sub>)—to environmental conditions that simulated “a growth period (Summer, April 17–September 15) followed by hardening (Fall, September 16–November 5; Winter, November 6–February 5) and dehardening (Spring, February 6–February 28).” During these periods numerous plant properties and physiological processes were measured.

In October, during the process of hardening, Bigras and Bertrand found “bud set began earlier in elevated CO<sub>2</sub> than in ambient CO<sub>2</sub>” and “an increase in seedling cold tolerance in early fall was related to early bud set in elevated CO<sub>2</sub>.” In addition, their results “showed reductions in dark respiration and light compensation point during cold acclimation and de-acclimation can contribute to increased productivity in elevated CO<sub>2</sub>.” They “observed an increase in light-saturated photosynthetic rate in response to elevated CO<sub>2</sub> during the growth, hardening and de-hardening periods” which “varied seasonally between 12 and 50%.” The net result of these and other CO<sub>2</sub>-induced plant benefits was a 38% increase in total plant mass at the end of the growing season that slowly declined to a value of 18% at the end of the winter.

Bertrand *et al.* (2007) grew well-watered and fertilized alfalfa (*Medicago sativa* L.) plants which they inoculated with one of two strains (either A2 or NRG34) of the nitrogen-fixing symbiont *Sinorhizobium meliloti* from seed in 12.5-cm-diameter pots filled with non-sterile topsoil within controlled-environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 400 or 800 ppm for two months under optimal light (600 μmol/m<sup>2</sup>/s for 16 hours per day) and day/night temperatures of 22/17°C and for two final weeks at a reduced light level of 200 μmol/m<sup>2</sup>/s for 8 hours per day and a cold day/night temperature regime of 5/2°C. Over this time they periodically measured plant physiological functions and characteristics.

At the end of the experiment, the researchers determined the total biomass of the plants in the elevated CO<sub>2</sub> treatment was approximately 33% greater than that of the plants in the control treatment when infected with the A2 strain of *S. meliloti*, but about 36% greater when infected with the NRG34 strain. Plants in the 800 ppm CO<sub>2</sub> treatment were found to be less freezing-tolerant than those in the 400 ppm treatment, and the plants inoculated with the NRG34 strain were determined to be less freezing-tolerant than those inoculated with the A2 strain.

Providing some comparative background for their

freezing tolerance results, Bertrand *et al.* write, “CO<sub>2</sub> enrichment led to more severe frost damage in leaves of *Eucalyptus pauciflora* (Barker *et al.*, 2005) and *Ginkgo biloba* (Terry *et al.*, 2000), and in a native temperate grassland (Obrist *et al.*, 2001), whereas it increased frost resistance of *Betula allaghamensis* (Wayne *et al.*, 1998) and *Picea mariana* (Bigras and Bertrand, 2006) but had no effect on freezing tolerance of *Picea abies* (Dalen *et al.*, 2001).” This suggests there may not be a single freezing tolerance response to atmospheric CO<sub>2</sub> enrichment that is typical of plants in general. However, because their results suggest “it is possible to select or identify rhizobial strains to improve alfalfa performance under high CO<sub>2</sub>,” Bertrand *et al.* conclude the “freezing tolerance as well as the expression of key overwintering genes of alfalfa can be altered by the strain of rhizobium.” This should enable farmers to obtain the best of both worlds, as it were, by benefiting from the significant growth stimulation produced by the ongoing rise in the air’s CO<sub>2</sub> content while selecting a strain of rhizobium capable of compensating for a possible CO<sub>2</sub>-induced reduction in freezing tolerance.

Hanninen *et al.* (2007) note “according to the hypothesis presented by Cannell (1985), climatic warming may paradoxically increase the risk of frost damage in boreal and temperate regions, because the trees will deharden and even start to grow during intermittent mild periods in winter and get damaged during subsequent periods of frost.” Likewise, they write, “Kellomaki *et al.* (1995) predicted that frost damage caused by a premature dehardening would severely restrict growth of Scots pine under a climatic warming scenario in central Finland.”

Hanninen *et al.* investigated this subject using whole-tree chambers (WTCs) surrounding groups of 40-year-old Norway spruce (*Picea abies* (L.) Karst.) trees growing under natural conditions in northern Sweden, studying the effects of atmospheric CO<sub>2</sub> enrichment (to 700 ppm above the ambient value of 365 ppm) and global warming (ranging from 2.8°C above ambient in summer to 5.6°C above ambient in winter) on the timing of spring bud burst in the trees.

The researchers report the timing of bud burst was unaffected by elevated CO<sub>2</sub>, but “the trees growing at an elevated temperature hardened later and dehardened earlier than the control trees.” However, they note, the difference was “much smaller” than that implied by Cannell (1985) and predicted by Kellomaki *et al.* (1995). Hanninen *et al.* conclude, “regardless of the warming taking place during winter, boreal coniferous trees are able to retain their dormancy and frost hardiness until around the spring

equinox,” explaining, “high temperatures during bud dormancy induction increase the chilling requirement of rest completion, or in some other way delay bud burst during spring,” citing Heide (2003) and Junttila *et al.* (2003). These observations thus suggest, they write, “boreal trees may be able to prevent premature dehardening and growth onset under climatic warming,” thereby thwarting scenarios of deleterious frost damage in the face of global warming.

In a study focusing only on temperature, Loik *et al.* (2004) note a warming of the climate may cause plants to come out of winter dormancy earlier in the spring, and “because physiologically active plants are more vulnerable than dormant plants to a transient low-temperature event (Larcher, 1995), earlier acclimation to warmer temperatures and onset of photosynthetic activity ... may make plants more susceptible to damage [caused by temporary but extreme cold weather] that reduces overall functional leaf surface area and productivity.” They tested this hypothesis for specimens of the evergreen shrub *Artemisia tridentata* and the sub-alpine herbaceous *Erythronium grandiflorum* that had been growing at 3,050 meters in the Rocky Mountains of Colorado (USA), “where overhead infrared heaters have continually simulated atmospheric forcing of the future (Harte *et al.*, 1995),” enhancing the downward infrared radiation flux by 22 Wm<sup>-2</sup>.

Specifically, they compared the photosynthetic responses of the plants before, during, and after an imposed *in situ* freezing event, where air and leaf temperatures of selected plants were “continually decreased, as would occur during a natural episodic freezing event.” This environmental manipulation started at an ambient temperature of about 10°C and adjusted to -30°C over a period of 10 hours, mimicking what occurs naturally at this site about once every 10 years.

The authors report “the infrared warming treatment did not reduce the ability of the subalpine herbaceous geophyte *Erythronium grandiflorum* to tolerate *in situ* freezing, in contrast to our hypothesis.” The warming treatment in fact “led to a significant increase in photosynthetic tolerance of an experimentally imposed *in situ* freezing event for the Great Basin Desert evergreen shrub *Artemisia tridentata* ... in contrast to our hypothesis.” Loik *et al.* suggest “enhanced tolerance of episodic freezing is a result of enhanced physiological activity, in particular, higher plant water potentials and photosynthetic gas exchange caused by the infrared treatment effects on soil water content and soil temperature,” leading to “an increased level of

photosynthates available for allocation to cryo-protection or other acclimation mechanisms (Iba, 2002).” Loik *et al.* thus conclude that, contrary to prior expectations, some species of plants will not be negatively affected by episodic freezing events in a warmer world, and others “may exhibit enhanced tolerance of subzero air temperatures under a future warmer climate.”

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## 2.14 Monoterpenes

- The diverse results of several experimental studies do not paint a clear picture of what should be expected in the way of plant monoterpene emissions in a CO<sub>2</sub>-enriched and possibly warmer world of the future.

Monoterpenes constitute a major fraction of the biogenic volatile organic compounds or BVOCs given off by plants; they help protect Earth's terrestrial vegetation by acting as scavengers of reactive oxygen species produced within plants experiencing significant heat stress (Peñuelas and Llusia, 2003). They also function as deterrents of pathogens and herbivores and are known to aid wound-healing after herbivore damage (Pichersky and Gershenzon, 2002). In addition, monoterpenes may attract pollinators and herbivore predators (Peñuelas *et al.*, 1995; Shulaeva *et al.*, 1997), and they have the ability to generate large quantities of organic aerosols that may alter the planet's climate by producing cloud condensation nuclei that can lead to a cooling of Earth's surface (during the day) via an enhanced reflection of incoming solar radiation.

In light of these observations, it is natural to want to know how plant monoterpene production might be affected by the ongoing rise in the atmosphere's CO<sub>2</sub> concentration, as well as by any warming that might yet occur as Earth continues to recover from the global chill of the Little Ice Age. What follows is a review of the findings of studies that have investigated various aspects of this subject.

Vuorinen *et al.* (2004) grew well-watered and fertilized white cabbage plants from seed for 25 days in growth chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 360 or 720 ppm. One group of plants in each CO<sub>2</sub> treatment experienced no larval insect feeding, another experienced 48 hours of

feeding by larvae of a crucifer specialist (*Plutella xylostella*), and yet another group experienced 48 hours of feeding by larvae of a generalist herbivore (*Spodoptera littoralis*), after which several BVOCs released by each group of plants were collected from the air surrounding them and analyzed. The researchers conclude, "total monoterpene emission per shoot dry weight was approximately 27% reduced from plants grown at elevated CO<sub>2</sub>," and they report there was no difference in larval-induced damage to the plants between the ambient-air and CO<sub>2</sub>-enriched treatments.

An earlier study of holly oak trees by Loreto *et al.* (2001) also found decreases in monoterpene emissions in response to atmospheric CO<sub>2</sub> enrichment, and Constable *et al.* (1999) found no effect of elevated CO<sub>2</sub> on monoterpene emissions from Ponderosa pine and Douglas fir trees. This may mean the effects of atmospheric CO<sub>2</sub> enrichment on plant monoterpene emissions are nil or even negative.

Baraldi *et al.* (2004) exposed sections of a southern California chaparral ecosystem to atmospheric CO<sub>2</sub> concentrations ranging from 250 to 750 ppm in 100-ppm increments for four years within naturally lit glass chambers, measuring net ecosystem CO<sub>2</sub> exchange (NEE) and emission rates of BVOCs, which were mainly monoterpenes. The seven scientists found NEE exhibited a marked linear increase in response to increasing atmospheric CO<sub>2</sub> concentration, more than tripling its rate in going from 400 to 700 ppm at 0400, 1200, and 1600 hours in June, and rising from moderately negative to weak positive values in December. They found "total trace gas emissions expressed on a ground area basis were low and did not respond to increasing CO<sub>2</sub> concentrations" in the winter; in summer, when "BVOC emissions were of an order of magnitude greater than during winter," they found the different levels of CO<sub>2</sub> still "did not affect the emission rates" of monoterpenes.

Rapparini *et al.* (2004) measured BVOC emissions from mature downy and holly oak trees growing close to a natural CO<sub>2</sub> spring in central Italy, where atmospheric CO<sub>2</sub> concentrations averaged about 1,000 ppm, and at a nearby control site where the air's CO<sub>2</sub> content was unaffected by the spring. They too found long-term exposure to high levels of atmospheric CO<sub>2</sub> did not significantly affect BVOC emissions from the trees. However, when leaves of plants grown in the control site were exposed for a short period to an elevated CO<sub>2</sub> level by rapidly switching the CO<sub>2</sub> concentration in the gas-exchange cuvette, monoterpene basal emissions "were clearly

inhibited,” where basal emissions are those that occur at standard measuring conditions of 30°C air temperature and 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light intensity.

The studies thus appear to suggest atmospheric CO<sub>2</sub> enrichment has little or no effect on plant monoterpene emissions, and that if there is any effect at all, it is negative. But the situation is not nearly that straightforward, as demonstrated by Staudt *et al.* (2001). These four researchers also grew holly oak, albeit holly oak seedlings, within two compartments of a controlled-environment greenhouse—one of which was maintained at an atmospheric CO<sub>2</sub> concentration of 350 ppm and one of which was maintained at 700 ppm—where air temperature and vapor pressure deficit were set to track outside ambient conditions and the plants were exposed to natural sunlight and watered every other week. Various growth parameters and physiological responses of the seedlings were measured over a four-month period that began when the trees had been exposed to the two CO<sub>2</sub> treatments for a total of 10 months. They report the elevated CO<sub>2</sub> treatment increased the leaf area of the young oaks by 40%, their leaf biomass by 50%, and their trunk and branch biomass by 90%. The plants in the elevated CO<sub>2</sub> treatment “released 2.8-fold more monoterpenes per plant than plants grown in ambient CO<sub>2</sub>,” on top of which Peñuelas and Llusia (2003) state, warming “increases the emission rates of most BVOCs exponentially.”

Raisanen *et al.* (2008) studied to what extent a doubling of the air’s CO<sub>2</sub> content and 2–6°C increases in air temperature (+2°C in summer, +4°C in spring and autumn, +6°C in winter), applied singly or together, might impact emissions of monoterpenes from 20-year-old Scots pine seedlings. They constructed closed-top chambers over parts of a naturally seeded stand of the trees in eastern Finland, which they exposed to these treatments for five years. Over the five-month growing season of May–September, the three researchers found total monoterpene emissions in the elevated-CO<sub>2</sub>-only treatment were 5% greater than those in the ambient-CO<sub>2</sub>-ambient-temperature treatment, and emissions in the elevated-temperature-only treatment were 9% less than those in ambient air. In the presence of both elevated CO<sub>2</sub> and elevated temperature, there was an increase of fully 126% in the total amount of monoterpenes emitted over the growing season.

The diverse results of these studies do not paint a clear picture of what should be expected in the way of plant monoterpene emissions in a CO<sub>2</sub>-enriched and possibly warmer world of the future.

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## 2.15 Nectar

- As the air’s CO<sub>2</sub> content continues to rise, plant

fitness, flower pollination, and nectar production should be enhanced, leading to increases in fruit, grain, and vegetable yields of agricultural crops, as well as similar increases in the productivity of the world's natural vegetation.

Lake and Hughes (1999) grew nasturtiums (*Tropaeolum majus*) from seed through flowering and senescence (77 days) in growth chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 380 or 760 ppm to determine the effects of elevated CO<sub>2</sub> on the vegetative and reproductive growth of the flowers, as well as several of the flowers' characteristics, including nectar quantity and quality. They found the doubled CO<sub>2</sub> concentration increased total plant biomass by 35% and root biomass by 78%, and it increased nectar volume in the CO<sub>2</sub>-enriched flowers by 2.4-fold. It did so without lowering the sugar and amino acid characteristics of the nectar.

Dag and Eisikowitch (2000) divided a 0.5-acre greenhouse located in the center of the Arava Valley in the southern part of Israel into two parts, one of which was exposed to ambient air and one of which was exposed to air that had a CO<sub>2</sub> concentration of 1,000 ppm throughout the morning, 400 ppm between 1300 and 1500 hours, and 600 ppm until the next morning. Under these conditions they grew melons (*Cucumis melo*). In the melons' early flowering stage, the scientists collected and measured the volume of nectar produced per flower between 0900 and 1530 hours along with the sugar concentration of the nectar. They found average nectar volumes per flower were significantly higher in the CO<sub>2</sub>-enriched sector of the greenhouse than in the control sector, sometimes by as much as 100%; since the sugar concentration of the nectar was found to be the same in both treatments, sugar production per flower was stimulated by an identical amount (as much as 100%) in the CO<sub>2</sub>-enriched air. As a result, and noting the only pollinator used in greenhouse production of melons in Israel is the honey bee, the two researchers conclude, "improvement in nectar reward can increase the attractiveness of the flowers to the bees, increase pollination activity and consequently increase the fruit set and the yield."

Erhardt *et al.* (2005) grew well-watered *Epilobium angustifolium* L. plants (perennial temperate clonal herbs that colonize nutrient-rich open habitats) from the seeds of five genotypes in pots containing 12 liters of loamy soil maintained at high and low levels of nutrients by weekly supplying them with 25 ml of either 1.0 N (high level) or 0.5 N (low level) Hoagland's solution. The experiment

lasted from April 1995 to July 1996 (two full growing seasons) and was conducted in naturally lit controlled environment chambers housed within a greenhouse. Half of the chambers were supplied with ambient air having a CO<sub>2</sub> concentration of about 350 ppm and half were supplied with CO<sub>2</sub>-enriched air having a concentration of about 650 ppm. Under these conditions, and in the second year of the study when most of the plants were flowering, nectar was extracted from the flowers, and its volume and sugar concentration were determined, along with its amino acid concentration and the total amino acid content per flower. The researchers found "elevated CO<sub>2</sub> significantly increased nectar production per day (+51%,  $p < 0.01$ ), total sugar per flower (+41%,  $p < 0.05$ ), amino acid concentration (+65%,  $p < 0.05$ ) and total amino acids per flower (+192%,  $p < 0.001$ )." These responses occurred with all genotypes.

Erhardt *et al.* note Galen and Plowright (1985) found "increased nectar rewards led to longer bumblebee tenure on flowers and greater pollen receipt in *E. angustifolium*, and that bees visited more flowers per plant on plants with more nectar." In addition, they report, "in other plant species higher nectar rewards also usually led to increases in components of plant fitness," citing Thomson (1986), Mitchell and Waser (1992), Mitchell (1993), Hodges (1995), and Irwin and Brody (1999).

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## 2.16 Nitrogen Fixation

Increases in the air's CO<sub>2</sub> content likely will stimulate nitrogen fixation in most herbaceous species that form symbiotic relationships with nitrogen-fixing soil bacteria. This phenomenon likely will lead to increased nitrogen availability in soils and large increases in both agricultural and natural ecosystem productivity. Increases in the air's CO<sub>2</sub> content also will greatly enhance the growth of Earth's leguminous trees, stimulating their fixation of nitrogen and their exudation of the resultant nitrogenous substances to the soils in which they grow, where they become available to neighboring non-leguminous vegetation.

### 2.16.1 Herbaceous Plants

- Increases in the air's CO<sub>2</sub> content likely will stimulate nitrogen fixation in most legumes, herbaceous species that form symbiotic relationships with nitrogen-fixing soil bacteria. This phenomenon likely will lead to increased nitrogen availability in soils, ultimately leading to large CO<sub>2</sub>-induced increases in agricultural and natural ecosystem productivity.

Nearly all of Earth's plants respond to increases in the air's CO<sub>2</sub> content by exhibiting increased rates of photosynthesis and biomass production. Additionally, most leguminous species—i.e., those that form symbiotic relationships with nitrogen-fixing bacteria—also find their ability to acquire nitrogen to be enhanced when exposed to CO<sub>2</sub>-enriched air. This phenomenon could increase their positive growth responses to elevated CO<sub>2</sub> even more and ultimately make more soil nitrogen available to co-occurring species. This section reviews several scientific studies of these phenomena that have been conducted on herbaceous plants.

Luscher *et al.* (1998) exposed several different grassland species to elevated levels of atmospheric CO<sub>2</sub> and observed nitrogen-fixing species tend to produce more biomass than non-nitrogen-fixing species, possibly in response to CO<sub>2</sub>-induced increases in the nitrogenase activity of symbiotic nitrogen-fixing bacteria associated with their roots. Dakora and Drake (2000) found a 300-ppm increase in the air's CO<sub>2</sub> concentration increased nitrogenase activity in a C<sub>3</sub> and a C<sub>4</sub> wetland species by 35 and 13%, respectively. Marilley *et al.* (1999) found enriching the air with CO<sub>2</sub> increased the dominance of nitrogen-fixing *Rhizobium* bacterial species associated with the roots of white clover. Arnone (1999), however, reported atmospheric CO<sub>2</sub> enrichment had no effect on symbiotic nitrogen fixation in *Trifolium alpinum*, a grassland species common to the Swiss Alps.

In a FACE study conducted on lucerne, plants fumigated with air containing 600 ppm CO<sub>2</sub> significantly increased their total tissue nitrogen content derived from symbiotic nitrogen-fixation (Luscher *et al.*, 2000). Plants grown on soil containing high nitrogen nearly doubled their symbiotically derived tissue nitrogen content, which rose from 21 to 41%, and plants grown on soils containing low nitrogen increased their symbiotically derived nitrogen content from 82 to 88%. In a related study performed on the same species, a doubling of the air's CO<sub>2</sub> content increased root nodule biomass by 40 and 100% in well-watered and water-stressed plants, respectively, as the CO<sub>2</sub>-enriched plants obtained 31 and 97% more total nitrogen than control plants under the same conditions (De Luis *et al.* 1999).

Lee *et al.* (2003b) investigated the effects of atmospheric CO<sub>2</sub> concentration (365 and 700 ppm) and nitrogen fertilization (low-N field soil + 0, 4, 8, 12, 16 and 20 g N m<sup>-2</sup> year<sup>-1</sup>) on leaf net photosynthesis, whole plant growth, and carbon and nitrogen acquisition in the N<sub>2</sub>-fixing wild lupine (*Lupinus perennis*) in controlled-environment chambers, where plants were grown from seed in pots for one full growing season. The mean rate of leaf net photosynthesis in the CO<sub>2</sub>-enriched chambers was 39% greater than in the ambient-air chambers, irrespective of N treatment, and total plant biomass at final harvest was 80% greater in the CO<sub>2</sub>-enriched chambers, again irrespective of N treatment. Elevated CO<sub>2</sub> increased plant total N by 57%, with the extra N coming from enhanced symbiotic N<sub>2</sub> fixation related to an increased number and overall mass of nodules. Although partial photosynthetic acclimation to CO<sub>2</sub>

enrichment occurred, the four researchers report the plants maintained significantly higher rates of photosynthesis and more efficient carbon capture per unit leaf N (average + 60%) in elevated CO<sub>2</sub> compared to ambient CO<sub>2</sub>, indicating a substantial CO<sub>2</sub>-induced increase in nitrogen use efficiency.

In mixed species experiments, Niklaus *et al.* (1998) found artificially constructed calcareous grassland swards were considerably more responsive to CO<sub>2</sub>-enriched air when legumes were present than when they were absent. In addition, they found elevated CO<sub>2</sub> stimulated nitrogen fixation, particularly when soil phosphorus was not limiting to growth. Thus, under conditions of adequate soil phosphorus, symbiotically derived nitrogen likely would become available for the use of non-nitrogen-fixing species.

Lee *et al.* (2003a) grew the N<sub>2</sub>-fixing *Lupinus perennis* in monoculture and in nine-species plots exposed to ambient air and air enriched to 560 ppm CO<sub>2</sub>. The proportion of *Lupinus* N derived from symbiotic N<sub>2</sub> fixation in monoculture increased from 44% in ambient air to 57% in CO<sub>2</sub>-enriched air, and in the nine-species plots it increased from 43% in ambient air to 54% in CO<sub>2</sub>-enriched air. Combined with the CO<sub>2</sub>-induced increases in plant biomass production, this resulted in a doubling of N fixed per plot under elevated compared to ambient CO<sub>2</sub>. Hartwig *et al.* (2002) obtained a similar result, observing a 70% increase in the air's CO<sub>2</sub> concentration to roughly double the amount of nitrogen input through symbiotic N<sub>2</sub>-fixation by white clover in a clover-ryegrass mixed ecosystem.

These findings indicate increases in the air's CO<sub>2</sub> content likely will stimulate nitrogen fixation in most legumes, and this phenomenon likely will lead to increased nitrogen availability in soils, ultimately leading to increases in agro- and natural ecosystem productivity.

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### 2.16.2 Woody Plants

- Continued increases in the air's CO<sub>2</sub> content will greatly enhance the growth of Earth's leguminous trees, stimulate their fixation of nitrogen, and increase their root exudations of nitrogenous substances to the soils in which they grow, where they become available for uptake by neighboring non-leguminous vegetation.

Nearly all of Earth's plants respond to increases in the air's CO<sub>2</sub> content by exhibiting enhanced rates of photosynthesis and biomass production. In addition, leguminous species possess the special ability to form symbiotic relationships with nitrogen-fixing bacteria, which can indirectly increase plant responses to elevated CO<sub>2</sub> by making more nitrogen available for plant uptake and utilization. This review summarizes the results of some of the studies that report the

effects of atmospheric CO<sub>2</sub> enrichment on these phenomena in woody plants.

Olesniewicz and Thomas (1999) grew black locust (*Robinia pseudoacacia*) seedlings for approximately two months in controlled environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 710 ppm, determining the elevated CO<sub>2</sub> increased total plant biomass by 180%. In addition, the extra CO<sub>2</sub> increased nitrogen-fixation by 69%, nodule mass by 92%, and the amount of seedling nitrogen derived from nitrogen-fixation by 212%. Working with the same species under much the same conditions, Uselman *et al.* (1999) determined between 1 and 2% of the total symbiotically fixed nitrogen is exuded from the tree's roots to become available for uptake by neighboring vegetation.

Schortemeyer *et al.* (1999) grew seedlings of *Acacia melanoxylon*, a leguminous nitrogen-fixing tree native to south-eastern Australia, for six weeks in growth cabinets maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm in hydroponic solutions with nitrogen concentrations ranging from 3 to 6,400 mmol m<sup>-3</sup>. Although atmospheric CO<sub>2</sub> enrichment did not stimulate symbiotic nitrogen fixation, averaged across all nitrogen treatments the seedlings grown in elevated CO<sub>2</sub> displayed net photosynthetic rates 22% higher than those of control seedlings, and they did not exhibit any signs of photosynthetic acclimation. These positive responses likely contributed to the doubled final biomass observed in the CO<sub>2</sub>-enriched seedlings in all but the two lowest nitrogen concentrations, where final biomass was unaffected by elevated CO<sub>2</sub>.

In a subsequent study of seven *Acacia* species native to Australia, Schortemeyer *et al.* (2002) once again grew seedlings in environmental chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm, but this time for nearly five months. They found the elevated CO<sub>2</sub> enhanced rates of net photosynthesis by 19 to 56% among all species and led to an average total plant dry weight increase of 86%. In addition, the elevated CO<sub>2</sub> increased the total amount of nitrogen fixed per plant by an average of 65%.

Temperton *et al.* (2003) measured total biomass and a number of physiological processes of N<sub>2</sub>-fixing *Alnus glutinosa* or common alder trees, which were grown for three years (1994–1996) in open-top chambers maintained at either ambient or elevated (ambient + 350 ppm) concentrations of atmospheric CO<sub>2</sub> and two soil nitrogen regimes (full nutrient solution or no fertilizer). The six scientists measured

nitrogen fixation by *Frankia* spp. in the root nodules of the trees, finding nitrogenase activity was consistently higher in the elevated CO<sub>2</sub> treatment in both 1995 and 1996. In addition, they report, “in October 1996, elevated CO<sub>2</sub> had a significant effect on total nodule dry mass, and there was a trend toward heavier nodules in the elevated CO<sub>2</sub> treatment than in the ambient CO<sub>2</sub> treatment.” They conclude, “most single-species studies on the effect of elevated CO<sub>2</sub> on N<sub>2</sub>-fixing species have reported stimulation of growth, nodule mass and nitrogenase activity (Norby, 1987; Arnone and Gordon, 1990; Hibbs *et al.*, 1995; Vogel and Curtis, 1995; Tissue *et al.*, 1997; Vogel *et al.*, 1997; Thomas *et al.*, 2000),” which is similar to what they observed.

These findings indicate continued increases in the air's CO<sub>2</sub> content will enhance the growth of Earth's leguminous trees, stimulate their fixation of nitrogen, and increase their root exudations of nitrogenous substances.

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## 2.17 Nitrogen Use Efficiency

- As the air's CO<sub>2</sub> content continues to rise, Earth's plants likely will reduce the amount of nitrogen invested in rubisco and other photosynthetic proteins while maintaining enhanced rates of photosynthesis and thereby increasing their photosynthetic nitrogen-use efficiencies.

Long-term exposure to elevated atmospheric CO<sub>2</sub> concentrations often, but not always, elicits photosynthetic acclimation or down-regulation in plants, which is typically accompanied by reduced amounts of rubisco and/or other photosynthetic proteins typically present in excess amounts in plants grown in ambient air. As a consequence, foliar nitrogen concentrations often decrease with atmospheric CO<sub>2</sub> enrichment, as nitrogen is mobilized out of leaves and into other areas of the plant to increase its availability for enhancing sink development or stimulating other nutrient-limited processes.

In reviewing the scientific literature on this subject, one quickly notices that even though photosynthetic acclimation has occurred, CO<sub>2</sub>-enriched plants still nearly always display rates of photosynthesis greater than those of control plants exposed to ambient air. Consequently, photosynthetic nitrogen-use efficiency—the amount of carbon

converted into sugars during the photosynthetic process per unit of leaf nitrogen—often increases in CO<sub>2</sub>-enriched plants.

Davey *et al.* (1999), for example, found CO<sub>2</sub>-induced reductions in foliar nitrogen contents and concomitant increases in photosynthetic rates led to photosynthetic nitrogen-use efficiencies in the CO<sub>2</sub>-enriched (to 700 ppm CO<sub>2</sub>) grass *Agrostis capillaris* 27 and 62% greater than those observed in control plants grown at 360 ppm CO<sub>2</sub> under moderate and low soil nutrient conditions, respectively. Similarly, they found elevated CO<sub>2</sub> enhanced photosynthetic nitrogen-use efficiencies in *Trifolium repens* by 66 and 190% under moderate and low soil nutrient conditions, respectively, and in *Lolium perenne* by 50%, regardless of soil nutrient status. Other researchers report similar CO<sub>2</sub>-induced enhancements of photosynthetic nitrogen-use efficiency in wheat (Osborne *et al.*, 1998) and in *Leucadendron* species (Midgley *et al.*, 1999).

In some cases, researchers report nitrogen-use efficiency in terms of the amount of biomass produced per unit of plant nitrogen. Niklaus *et al.* (1998), for example, report intact swards of CO<sub>2</sub>-enriched calcareous grasslands grown at 600 ppm CO<sub>2</sub> attained total biomass values 25% greater than those of control swards exposed to ambient air while extracting the same amount of nitrogen from the soil as ambiently grown swards. Deng and Woodward (1998) reported similar results for strawberry, noting the growth-based nitrogen-use efficiencies of plants grown at 560 ppm CO<sub>2</sub> were 23 and 17% greater than those of ambiently grown plants simultaneously subjected to high and low soil nitrogen availability, respectively.

The scientific literature suggests as the air's CO<sub>2</sub> content continues to rise, Earth's plants likely will respond by reducing the amount of nitrogen invested in rubisco and other photosynthetic proteins while maintaining enhanced rates of photosynthesis, which should increase their photosynthetic nitrogen-use efficiencies. As overall plant nitrogen-use efficiency increases, plants likely will grow ever better on soils containing less-than-optimal levels of nitrogen.

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## 2.18 Nutrient Acquisition

- As the CO<sub>2</sub> content of the air increases, much of Earth's vegetation likely will display increases in biomass. The larger plants likely will develop more extensive root systems and extract greater amounts of mineral nutrients from the soils in which they are rooted, enabling them to sustain their enhanced growth.

With respect to acquiring nitrogen, Zak *et al.* (2000) found aspen seedlings grown for 2.5 years at twice-ambient CO<sub>2</sub> concentrations displayed an average total seedling nitrogen content 13% greater than that displayed by control seedlings grown in ambient air, in spite of an average reduction in tissue nitrogen concentration of 18%. Elevated CO<sub>2</sub> enhanced total nitrogen uptake from the soil, even though tissue nitrogen concentrations in the CO<sub>2</sub>-enriched plants were diluted by the enhanced biomass of the much larger CO<sub>2</sub>-enriched seedlings.

On a per-unit-biomass basis, Smart *et al.* (1998) note there were no differences in the total amounts of nitrogen within CO<sub>2</sub>-enriched and ambiently grown wheat seedlings after three weeks of exposure to atmospheric CO<sub>2</sub> concentrations of 360 and 1,000 ppm. Nevertheless, the CO<sub>2</sub>-enriched seedlings exhibited greater rates of soil nitrate extraction than the ambient-grown plants did.

Similarly, BassiriRad *et al.* (1998) report a doubling of the atmospheric CO<sub>2</sub> concentration doubled the uptake rate of nitrate in the C<sub>4</sub> grass *Bouteloua eriopoda*. However, they also report elevated CO<sub>2</sub> had no effect on the rate of nitrate uptake in *Prosopis*, and it actually decreased the rate of nitrate uptake by 55% in *Larrea*. Nonetheless,

atmospheric CO<sub>2</sub> enrichment increased total biomass in these two species by 55 and 69%, respectively. Thus, although the uptake rate of this nutrient was depressed under elevated CO<sub>2</sub> conditions in the latter species, the much larger CO<sub>2</sub>-enriched plants likely still extracted more total nitrate from the soil than did the ambient-grown plants of the experiment.

Nasholm *et al.* (1998) determined trees, grasses, and shrubs can absorb significant amounts of organic nitrogen from soils. Thus, plants do not have to wait for the mineralization of organic nitrogen before they extract the nitrogen they need from soils to support their growth and development. As a result, the forms of nitrogen removed from soils by plants (nitrate vs. ammonium) and their abilities to remove different forms may not be as important as was once thought.

Finzi *et al.* (2002) found, on average, loblolly pine exposed to an extra 200 ppm of CO<sub>2</sub> maintained rates of net primary productivity 25% greater, and produced 32% more biomass, than trees growing in ambient air. The elevated CO<sub>2</sub> also increased the total amount of nitrogen present in the trees' biomass. The average annual requirement for nitrogen rose by 16% for the trees growing in the air enriched with CO<sub>2</sub>. To compensate for this increased nitrogen demand, the average uptake of nitrogen from the soil was enhanced by 28% in the CO<sub>2</sub>-enriched plot—which says a lot, considering the soils in the study region are characteristically low in available nitrogen. In addition, average nitrogen-use efficiency rose by approximately 10% with atmospheric CO<sub>2</sub> enrichment.

With respect to the uptake of phosphate, Staddon *et al.* (1999) report *Plantago lanceolata* and *Trifolium repens* plants grown at 650 ppm CO<sub>2</sub> for 2.5 months exhibited total plant phosphorus contents much greater than those displayed by plants grown at 400 ppm CO<sub>2</sub>, because atmospheric CO<sub>2</sub> enrichment significantly enhanced plant biomass. Similarly, Rouhier and Read (1998) report enriching the air around *Plantago lanceolata* plants with an extra 190 ppm of CO<sub>2</sub> for three months led to increased uptake of phosphorus and greater tissue phosphorus concentrations than were observed in plants growing in ambient air.

Greater uptake of phosphorus also can occur due to CO<sub>2</sub>-induced increases in root absorptive surface area or enhancements in specific enzyme activities. In addressing the first of these phenomena, BassiriRad *et al.* (1998) report a doubling of the air's CO<sub>2</sub> concentration significantly increased the belowground biomass of *Bouteloua eriopoda* and doubled its uptake rate of phosphate. However, elevated CO<sub>2</sub> had

no effect on uptake rates of phosphate in *Larrea* and *Prosopis*. Because the CO<sub>2</sub>-enriched plants grew so much bigger, however, they still removed more phosphate from the soil on a per-plant basis. With respect to the second phenomenon, activity of phosphatase—the primary enzyme responsible for the conversion of organic phosphate into usable inorganic forms—increased by 30 to 40% in wheat seedlings growing at twice-ambient CO<sub>2</sub> concentrations (Barrett *et al.*, 1998).

With respect to another nutrient, iron, Jin *et al.* (2009) grew 20-day-old plants for an additional seven days within controlled-environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 350 or 800 ppm in an iron (Fe)-sufficient medium with a soluble Fe source or under Fe-limited conditions in a medium containing the sparingly soluble hydrous Fe(III)-oxide. Their results indicate plant growth was increased by the elevated CO<sub>2</sub> in both the Fe-sufficient and Fe-limited media, with shoot fresh weight increasing by 22% and 44%, respectively, and root fresh weight increasing by 43% and 97%, respectively. In addition, Jin *et al.* report, “the elevated CO<sub>2</sub> under Fe-limited conditions enhance[d] root growth, root hair development, proton release, root FCR [ferric chelate reductase] activity, and expressions of *LeFR01* and *LeIRT1* genes [which respectively encode FCR and the Fe(II) transporter in tomato], all of which enable plants to access and accumulate more Fe.” They add, “the associated increase in Fe concentrations in the shoots and roots alleviated Fe-deficiency-induced chlorosis.”

Jin *et al.* note the bioavailability of iron to terrestrial plants “is often limited (Guerinot and Yi, 1994), particularly in calcareous soils, which represent 30% of the Earth’s surface (Imsande, 1998),” and thus conclude, “Fe nutrition in plants is likely to be affected by the continued elevation of atmospheric CO<sub>2</sub>, which, in turn, will affect crop production.” As their work strongly suggests, those important effects should be highly beneficial. Even wider biospheric benefits are suggested by the work of Sasaki *et al.* (1998), who demonstrated both the ferric reductase activity and Fe uptake capacity of the marine alga *Chlorococcum littorale* cultured in Fe-limited media have been significantly enhanced by elevated CO<sub>2</sub> concentrations.

Haase *et al.* (2008) grew barley (*Hordeum vulgare* L. cv. Europa) plants from seed for four weeks—both hydroponically in nutrient solution having adequate or less-than-adequate iron (Fe) concentrations (+Fe and -Fe, respectively), as well as in rhizobox microcosms filled with soil under the

same two conditions of iron availability—in controlled-environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 400 or 800 ppm. The elevated atmospheric CO<sub>2</sub> treatments stimulated biomass production in both Fe-sufficient and Fe-deficient barley plants, in both hydroponics and soil culture. In addition, they found three CO<sub>2</sub>-induced modifications in plant activity: “(i) increased internal Fe use efficiency, (ii) stimulation of root growth, and (iii) increased root exudation of Fe-mobilizing phytosiderophores in the sub-apical root zones.” Since phytosiderophores act as metal chelators that mobilize sparingly soluble inorganic forms of iron and zinc and make them more readily available to plants, the researchers suggest atmospheric CO<sub>2</sub> enrichment increases the competitiveness of plants such as barley with rhizosphere microorganisms in their quest for these often difficult-to-obtain trace elements. This phenomenon helps to explain the strong growth response of barley to atmospheric CO<sub>2</sub> enrichment even when iron availability is low.

In a study dealing with multiple nutrients, Schaffer *et al.* (1997) grew two mango ecotypes, one evolving from a warm, humid tropical climate and the other from a cool, dry subtropical region, for 12 months in glasshouses with atmospheres of either 350 or 700 ppm CO<sub>2</sub> to determine the effects of atmospheric CO<sub>2</sub> enrichment on plant growth and leaf mineral nutrient concentrations. According to the researchers, although atmospheric CO<sub>2</sub> enrichment led to partial photosynthetic acclimation in both ecotypes, greater net carbon gains were achieved with elevated CO<sub>2</sub>, as indicated by greater plant dry mass values for trees grown at 700 ppm CO<sub>2</sub>. Elevated CO<sub>2</sub> also tended to decrease foliar concentrations of mineral nutrients (N, P, K, Ca, Mg, S, Cl, Fe, Zn, Mn, Cu, and B) in both mango cultivars, most likely due to a dilution effect, since atmospheric CO<sub>2</sub> enrichment increased leaf dry mass. Although the instantaneous doubling of the atmospheric CO<sub>2</sub> concentration in this experiment reduced the concentrations of leaf mineral elements, Schaffer *et al.* state, “given the slow rate at which global atmospheric CO<sub>2</sub> concentration is increasing, it is possible that plants will adapt to elevated ambient CO<sub>2</sub> concentrations over time with respect to mineral nutrition,” as did sour orange trees after 85 months of exposure to elevated CO<sub>2</sub> (Peñuelas *et al.*, 1997).

Lieffering *et al.* (2004) analyzed the elemental concentrations of archived grain samples from temperate rice (*Oryza sativa* L. cv. Akitakomachi) crops grown previously under FACE conditions out-

of-doors in a fertile agricultural field (Okada *et al.*, 2001), where an approximate 200-ppm increase in the air's CO<sub>2</sub> concentration increased rice grain yields by about 14% (Kim *et al.*, 2003a,b).

Of the five macro-nutrients they measured (N, P, K, Mg, S), Lieffering *et al.* found “only N showed a decrease in concentration with elevated CO<sub>2</sub> in both years,” and all six of the micro-nutrients studied (Zn, Mn, Fe, Cu, B, Mo) exhibited concentration increases. For Zn and Mn, in particular, they state, “there was a strong tendency [for concentrations] to increase,” and the same could have been said of Fe, which in the second year of the study exhibited a CO<sub>2</sub>-induced concentration increase on the order of 68%, as best as can be determined from Lieffering *et al.*'s bar graphs.

Lieffering *et al.* note their study of the effects of elevated CO<sub>2</sub> on grain elemental concentrations under real-world field conditions is “the first such report for a staple food crop: all other previously reported data were obtained from plants growing in pots and in some kind of enclosure.” In contrast to the results obtained in most of these latter root-confining experiments, they note that, other than for N, “no dilution of [the] elements in the grain was observed, contrary to the general conclusions of Loladze (2002).” Therefore, they conclude, “as long as there is a readily available supply of nutrients and the nutrient uptake capacity response to elevated CO<sub>2</sub> is equal [to] or greater than the whole plant biomass response [which was the case in their experiment, except for N], then no dilution should be observed.”

Natali *et al.* (2009) noted “increased production of fine roots with CO<sub>2</sub> enrichment (Norby *et al.*, 2004; Pritchard *et al.*, 2008) may allow plants to match increased carbon assimilation with increased uptake of soil-derived elements.” They “examined CO<sub>2</sub> effects of a suite of metal micronutrients and contaminants in forest trees and soils at two free-air CO<sub>2</sub> enrichment sites—a loblolly pine forest in North Carolina (Duke) and a sweetgum plantation in Tennessee [Oak Ridge National Laboratory (ORNL)]—as well as at an open-top chamber experiment in a scrub-oak community in Florida [Smithsonian Environmental Research Center (SERC)].” They “did not find an overall decline in foliar metal concentrations with CO<sub>2</sub> enrichment,” but they *did* find dilution effects for metal micronutrients were generally “less than for non-essential trace metals,” and “some essential plant metals were greater under elevated CO<sub>2</sub> (for example, a 28% increase in Mn across species and sites).” Natali *et al.* conclude their results “should alleviate some concerns

that rising CO<sub>2</sub> concentrations will result in broad-scale decreases in the concentrations of all elements essential for plant function and animal nutrition,” as proposed by Loladze (2002). They also state their generally opposite results for nonessential trace elements (some of which can be toxic) “may be applicable to contaminated systems,” stating, “elevated CO<sub>2</sub> may, through dilution effects, alleviate aluminum toxicity.”

In a somewhat different type of study, Urabe and Waki (2009) grew three algal species—*Scenedesmus obliquus* (green algae), *Cyclotella* sp. (diatoms), and *Synechococcus* sp. (cyanobacteria)—in mono- and mixed-cultures at ambient (360 ppm) and high (2,000 ppm) CO<sub>2</sub> levels and allowed a planktonic herbivore (*Daphnia*) to feed on the different algal populations thereby produced either individually or in various mixtures, to see if there was any CO<sub>2</sub>-induced effect on herbivore growth. The two researchers found “both in the mono- and mixed cultures, algal steady state abundance increased but algal P:C and N:C ratios decreased when they were grown at high CO<sub>2</sub>.” They also found “*Daphnia* fed monospecific algae cultured at high CO<sub>2</sub> had decreased growth rates despite increased algal abundance.” But “when fed mixed algae cultured at high CO<sub>2</sub>, especially consisting of diatoms and cyanobacteria or the three algal species,” they found “*Daphnia* maintained high growth rates despite lowered P and N contents relative to C in the algal diets.” The findings indicate, they write, “algal diets composed of multiple species can mitigate the adverse effects of elevated CO<sub>2</sub> on herbivore performance,” and “in environments with high CO<sub>2</sub>, herbivores may find a new diet producer or a combination of producer species to best meet their nutritional demands.”

The experimental data accumulated to date suggest much of Earth's vegetation likely will display increases in biomass as the air's CO<sub>2</sub> content rises, and considerable evidence suggests the larger plants thereby produced will develop more extensive root systems and extract greater amounts of mineral nutrients from the soils in which they are rooted, enabling them to sustain their enhanced growth.

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## 2.19 Phenolics

Herbaceous plants often experience increases in foliar and fruit phenolic concentrations in response to atmospheric CO<sub>2</sub> enrichment, and these responses appear to have a number of positive implications for both man and the biosphere. Future increases in the air's CO<sub>2</sub> concentration likely will enhance foliar phenolic concentrations in many trees and shrubs, and this should enhance woody-plant defense mechanisms that help deter herbivory, thereby improving forest health, robustness, and longevity.

### 2.19.1 Herbaceous Species

- Many, but not all, herbaceous plants experience increases in foliar and fruit phenolic concentrations in response to atmospheric CO<sub>2</sub> enrichment. These responses appear to have a number of positive implications for both man and the biosphere.

In the presence of elevated concentrations of atmospheric CO<sub>2</sub>, many of Earth's plants display an enhanced rate of photosynthetic carbon uptake, which leads to an increased production of plant secondary carbon compounds, including phenolics. Since the resulting increases in plant foliar phenolic concentrations often enhance plant resistance to herbivore and pathogen attack, this section reviews the results of some studies that have dealt with this important subject in herbaceous plants.

Hoorens *et al.* (2002) grew two plants common to dune grasslands of the Netherlands (*Calamagrostis epigejos* and *Vicia lathyroides*), along with two species common to Dutch peatlands (*Carex rostrata* and *Sphagnum recurvum*), in greenhouses fumigated with air containing either 390 or 700 ppm CO<sub>2</sub> for five months. Then, after senescence had occurred, they collected the resulting leaf litter and analyzed it for the presence of various substances. Among other things, they found elevated CO<sub>2</sub> had little impact on the presence of phenolics in *Calamagrostis* and *Sphagnum* litter, but it increased phenolic concentrations in *Vicia* and *Carex* litter by 20 and 32%, respectively.

Castells *et al.* (2002) grew 14 genotypes of two perennial grasses common to the Mediterranean area (*Dactylis glomerata* and *Bromus erectus*) in glasshouses maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm in order to determine whether elevated CO<sub>2</sub> impacts phenolic

production in a genotypic-dependent manner. Their research indicates doubling the air's CO<sub>2</sub> concentration increased total phenolic concentrations in *Dactylis* and *Bromus* by 15 and 87%, respectively, and there were no significant CO<sub>2</sub> x genotype interactions in either species. As the atmosphere's CO<sub>2</sub> concentration continues to rise, therefore, these two perennial grasses likely will exhibit greater resistance to herbivory without having to sacrifice their genotypic diversity.

Wetzel and Tuchman (2005) grew cattails for three years in open-bottom root boxes out-of-doors within clear-plastic-wall open-top chambers maintained at either ambient (360 ppm) or elevated (720 ppm) atmospheric CO<sub>2</sub> concentrations from early spring through leaf senescence. During this period, green and naturally senesced leaves were collected and analyzed for the fraction of leaf mass composed of total phenolics. The researchers found green leaf material contained 27.6% more total phenolics when the plants were grown in CO<sub>2</sub>-enriched as opposed to ambient air, and senesced leaf material grown in CO<sub>2</sub>-enriched air contained 40.6% more total phenolics than similar leaves produced in ambient air.

In a study with human health implications, Wang *et al.* (2003) grew strawberry plants in six clear-acrylic open-top chambers, two of which were maintained at the ambient atmospheric CO<sub>2</sub> concentration, two of which were maintained at ambient + 300 ppm CO<sub>2</sub>, and two of which were maintained at ambient + 600 ppm CO<sub>2</sub> from early spring 1998 through June 2000. During this time, the researchers harvested the strawberry fruit, they write, "at the commercially ripe stage" in both 1999 and 2000. They analyzed the fruit for the presence of a number of different health-promoting substances with "potent antioxidant properties." They found CO<sub>2</sub> enrichment increased fruit ascorbic acid, glutathione, phenolic acid, flavonol, and anthocyanin concentrations, and plants grown under the CO<sub>2</sub> enriched conditions also had higher oxygen radical absorbance activity against many types of harmful oxygen radicals in the fruit.

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### 2.19.2 Woody Species

- Future increases in the air's CO<sub>2</sub> concentration likely will enhance foliar phenolic concentrations in many shrubs and trees. This, in turn, should enhance woody-plant defense mechanisms that help deter herbivory and thus improve forest health, robustness, and longevity.

As the air's CO<sub>2</sub> content continues to rise, many of Earth's plants are experiencing enhanced rates of photosynthetic carbon uptake, which commonly leads to increased production of plant secondary carbon compounds, including phenolics that often enhance plant resistance to herbivore and pathogen attack. This section examines the results of several studies dealing with this important subject.

Peñuelas *et al.* (2002) sampled leaves of three species of shrubs growing close to, and further away from, CO<sub>2</sub>-emitting springs in Pisa, Italy, to determine the long-term effects of elevated atmospheric CO<sub>2</sub> on foliar concentrations of carbon-based secondary compounds. The researchers found the extra 340 ppm of CO<sub>2</sub> near the springs had very few long-term significant effects on foliar concentrations of most such substances, including phenolics, and those effects that were observed varied according to specific compound and plant species. Their findings are somewhat atypical of what is often observed, as several studies of temperate-region trees have shown leaf phenolic concentrations to rise by 20 to 60% in response to a doubling of the air's CO<sub>2</sub> content (Koricheva *et al.*, 1998; Peñuelas and Estiarte, 1998; McDonald *et al.*, 1999; Agrell *et al.*, 2000; Hartley *et al.*, 2000), as also was reported by Parsons *et al.* (2003) with respect to the total fine-root phenolic concentrations of warm-temperate conifers studied by King *et al.* (1997), Entry *et al.* (1998), and Runion *et*

*al.* (1999).

Gebauer *et al.* (1998) grew loblolly pine seedlings in glasshouses maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm for five months while subjecting them to four levels of soil nitrogen fertilization. Across all of these nitrogen regimes, the extra CO<sub>2</sub> increased the above- and below-ground concentrations of seedling total phenolics by 21 and 35%, respectively.

Booker and Maier (2001) also worked with loblolly pines, measuring concentrations of total soluble phenolics in needles exposed for two years in branch chambers to ambient air and air enriched to as much as 350 ppm CO<sub>2</sub> above ambient. They found needle concentrations of total soluble phenolics increased about 11% in response to the elevated CO<sub>2</sub>, noting this response was related to “the balance between carbohydrate sources and sinks,” so “the greater the source:sink ratio, the greater the concentration of phenolic compounds (Herms and Mattson, 1992; Peñuelas and Estiarte, 1998).”

In contrast, Hamilton *et al.* (2004) studied the understory of the loblolly pine plantation in the Duke Forest FACE study, finding no evidence of significant changes in total leaf phenolics in either of two years, in agreement with the results of “another study performed at the Duke Forest FACE site that also found no effect of elevated CO<sub>2</sub> on the chemical composition of leaves of understory trees (Finzi and Schlesinger, 2002).” Nevertheless, they state, “elevated CO<sub>2</sub> led to a trend toward reduced herbivory in [the] deciduous understory in a situation that included the full complement of naturally occurring plant and insect species.” In 1999, for example, they found “elevated CO<sub>2</sub> reduced overall herbivory by more than 40% with elm showing greater reduction than either red maple or sweetgum,” and in 2000 they observed “the same pattern and magnitude of reduction.”

In a similar FACE study of an ecosystem dominated by three species of oak tree (*Quercus myrtifolia*, *Q. chapmanii*, and *Q. geminata*) plus the nitrogen-fixing legume *Galactia elliotii* at the Kennedy Space Center in Florida (USA), Hall *et al.* (2005) detected no significant differences between the CO<sub>2</sub>-enriched and ambient-treatment leaves of any single species in terms of condensed tannins, hydrolyzable tannins, total phenolics, or lignin. When all four species were considered together, however, there were always greater concentrations of all four leaf constituents in the CO<sub>2</sub>-enriched leaves, with across-species mean increases of 6.8% for condensed tannins, 6.1% for hydrolyzable tannins, 5.1% for total

phenolics, and 4.3% for lignin. In addition, there were large CO<sub>2</sub>-induced decreases in all leaf damage categories associated with herbivory: chewing (-48%,  $P < 0.001$ ), mines (-37%,  $P = 0.001$ ), eye spot gall (-45%,  $P < 0.001$ ), leaf tier (-52%,  $P = 0.012$ ), leaf mite (-23%,  $P = 0.477$ ), and leaf gall (-16%,  $P = 0.480$ ). The five researchers conclude the changes in leaf chemical constituents and herbivore damage “suggest that damage to plants may decline as atmospheric CO<sub>2</sub> levels continue to rise.”

Wetzel and Tuchman (2005) grew trembling aspen seedlings for three years in open-bottom root boxes out-of-doors within clear-plastic-wall open-top chambers maintained at either ambient (360 ppm) or elevated (720 ppm) atmospheric CO<sub>2</sub> concentrations from early spring through leaf senescence. Green and naturally senesced leaves were collected and analyzed for the fraction of leaf mass composed of total phenolics. Green leaf material contained 19.1% more total phenolics when the experimental seedlings were grown in CO<sub>2</sub>-enriched as opposed to ambient air, and senesced leaf material grown in CO<sub>2</sub>-enriched air contained 63.2% more total phenolics than similar leaf material grown in ambient air.

In Finland, Kuokkanen *et al.* (2003) grew two-year-old birch trees in the field in closed-top chambers exposed to either ambient air of 350 ppm CO<sub>2</sub> or air enriched to a CO<sub>2</sub> concentration of 700 ppm at either ambient temperatures or ambient temperatures plus 3°C for one full growing season. During the middle of summer, when carbon-based secondary compounds of birch leaves are usually fairly stable, they picked several leaves from each tree and measured the concentrations of a number of physiologically important substances. The concentration of total phenolics significantly increased in the birch leaves produced in the CO<sub>2</sub>-enriched air, as also has been observed in the experiments of Lavola and Julkunen-Titto (1994), Williams *et al.* (1994), Kinney *et al.* (1997), Bezemer and Jones (1998), and Kuokkanen *et al.* (2001).

Coley *et al.* (2002) studied nine species of tropical trees rooted in the ground and grown in their natural environment near the Smithsonian Tropical Research Institute's experiment site in central Panama. Their six-month open-top chamber study produced impressive results, with eight of the nine species exhibiting positive leaf phenolic/tannin responses to a doubling of the air's CO<sub>2</sub> content, the largest of which was a concentration increase of 119%. The single negative response was a 27% decline, and the mean response of all nine species was an increase of 48%. These results are comparable to

those obtained for temperate-region trees, and they provide the basis for Coley *et al.*'s primary conclusion: Although “both temperate and tropical trees show large interspecific variation in the extent of their response to CO<sub>2</sub> ... the overwhelming pattern is for an increase in phenolics by approximately 50%.”

The results of these several studies suggest future increases in the air's CO<sub>2</sub> concentration likely will enhance foliar phenolic concentrations in many shrubs and trees. This phenomenon, in turn, should enhance woody-plant defense mechanisms that help deter herbivory, which should improve forest health, robustness, and longevity.

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## 2.20 Phenology

- The wide range of results obtained by many past and current methods of detecting each year's start-of-spring has led some researchers to claim spring has been arriving earlier over the course of the twentieth century. These observations are used to support the associated claim of twentieth-century CO<sub>2</sub>-induced global warming, often said to result in the mistiming of the yearly start-up of the component links of various ecosystems' food chains. Real-world data, however, do not support the bulk of these contentions.

The progressively earlier arrival of spring with each succeeding year is widely viewed as an indicator of global warming. Scheifinger *et al.* (2002) and Schaber and Badeck (2005) claim to have detected earlier spring arrival in Europe over the course of the twentieth century. One difficulty with such studies, however, is the lack of a commonly agreed-upon metric for the arrival of spring.

White *et al.* (2009) compared 10 algorithms for processing satellite Normalized Difference Vegetation Index (NDVI) data for this purpose, based on the expectation that satellite data, with their continental coverage, should allow such trends to be more easily detected. A question that remains, however, is whether the various methods employed measure the same things or correspond to on-the-ground measurements.

White *et al.*'s study covered the period 1982–2006, using 16,000 ground-based phenological measurements from across North America as well as data on snow melt and hydrology. Using the 10 methods of extracting a start-of-spring (SOS) signal from the satellite data, they ended up with a range of fully 60 days around the 10-model mean estimate.

The 10 methods also tended to be biased toward detecting SOS earlier than ground-based phenology data indicated, and to have weak correlations with the ground data, perhaps because there were difficulties classifying pixels in some cases, particularly for desert, semi-tropical, and Mediterranean (California) zones in which the arrival of “spring” is not a well-defined phenomenon.

In the end, the 21 researchers state they “found no evidence for time trends in spring arrival from ground- or model-based data,” and “using an ensemble estimate from two methods that were more closely related to ground observations than other methods, SOS trends could be detected for only 12% of North America,” with 7% showing a trend to earlier spring and 5% exhibiting a trend toward later spring. That suggests the wide range of results obtained by most past and current methods of detecting the start of spring may have led to premature claims of spring’s increasingly earlier annual occurrence over the latter part of the twentieth century. And that finding, in turn, raises significant concerns about the validity of near-surface air temperature measurements that suggest North America has warmed significantly over the past several decades.

In the introduction to another study of the subject, however, Johnston and Reekie (2008) state there have “been marked changes in plant phenology over the past century,” and these changes “have been interpreted as a consequence of the increase in temperature that has been observed over this time.” In an added twist to the subject, they speculate, “the concentration of atmospheric CO<sub>2</sub> may also directly affect time of flowering, even in the absence of temperature change.”

The two researchers examined the effect of elevated atmospheric CO<sub>2</sub> concentration by itself (ambient and ambient + 330 ppm), as well as the combined effect of elevated CO<sub>2</sub> and elevated air temperature (ambient + 1.5°C), on the flowering phenology of 22 species of plants in the Asteraceae family, which were grown under natural, seasonally varying temperature and daylength in separate compartments of a glasshouse in Wolfville, Nova Scotia, Canada. The researchers found, “on average, elevated CO<sub>2</sub> by itself advanced flowering by four days” and “increasing temperature as well as CO<sub>2</sub> advanced flowering by an additional three days.” They also found, “CO<sub>2</sub> was more likely to hasten phenology in long- than in short-day species,” and “early- and late-flowering species did not differ in response to elevated CO<sub>2</sub>,” but “the combined effect

of elevated CO<sub>2</sub> and temperature hastened flowering more in early- than late-flowering species.” They conclude, with respect to time of flowering in Asteraceae species, “the direct effect of CO<sub>2</sub> on phenology may be as important as its indirect effect through climate change,” further complicating the interpretation of a progressively earlier start of spring as an indication of regional or global warming.

Some scientists express concern that global warming may “throw off” the timing of lifecycle stages of certain food chain components, leading to a mismatch among the unique needs of different ecosystem trophic levels that could spell disaster for some species. Visser and Both (2005) warn of such an “insufficient adjustment” to climate change.

Bauer *et al.* (2010) studied the effect of 47 years of warming (1961–2007) on the time of leafing-out of dominant English Oak (*Quercus robur*) trees at four research sites in the Czech Republic located in full-grown, multiaged floodplain forests that had been under no forestry management. They also studied the time of appearance of the two most abundant species of caterpillars in the floodplain forests, the Winter Moth (*Operophtera brumata*) and the Tortrix Moth (*Tortrix viridana*), and the first and mean laying dates of two of the ecosystem’s most common birds: Great Tits (*Parus major*) and Collared Flycatchers (*Ficedula albicollis*).

The seven scientists found “mean annual temperature showed a significant increase of 0.27–0.33°C per decade, with approximately the same magnitude of change during spring at all sites.” They also found, “on average (all four sites), the bud burst date for English Oak has advanced by 7.9 days and full foliage by 8.9 days, with approximately the same shifts being recorded for the peak of the beginning and end of frass for herbivorous caterpillars,” which was the observational variable they used to characterize the caterpillars’ presence. Finally, they write, “the first laying date of Great Tits has advanced by between 6.2 to 8.0 days,” and “the mean laying date has advanced by 6.4 to 8.0 days.” They found the “Collared Flycatcher first laying date has advanced by 8.5 to 9.2 days over the past 47 years, and the mean laying date by 7.7 to 9.6 days.”

Bauer *et al.* state, because “trends in the timing of reproduction processes of both bird species are coherent with the trends in development of English Oak and with peak herbivorous caterpillar activity,” it is apparent in this specific food chain the common temporal shifting of the different organisms’ phenological stages toward the beginning of the year “does not appear to have led to mistiming in the

trophic food chain.” Hence, other food chains also may not be as seriously disrupted by global warming as many have postulated they could be. More work of this nature, however, is clearly needed before any generalizations are warranted.

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### 2.21 Protein

- In both managed agricultural crops and the wild plants of Earth’s natural ecosystems, the ongoing rise of the air’s CO<sub>2</sub> concentration likely will have few negative impacts of any consequence on the nutritive value of their grains and foliage in terms of protein concentration. In tree crops such as citrus, CO<sub>2</sub>-induced changes in the activities of certain foliar proteins could lead to vast increases in yield potential.

In a review of the scientific literature on the effects of atmospheric CO<sub>2</sub> enrichment on plant constituents of significance to human health, Idso and Idso (2001) cite a number of studies where elevated levels of atmospheric CO<sub>2</sub> either increased, decreased, or had no effect on the protein concentrations of various agricultural crops. The first two of these consequences also were observed by Kaddour and Fuller (2004) and Veisz *et al.* (2005) in wheat.

In the case of this particular crop—which according to Wittwer (1995) was “the most widely grown plant in the world” at that time, contributing “more calories and protein to the human diet than any other food”—Pleijel *et al.* (1999) analyzed the results of 16 open-top chamber experiments that had been conducted on spring wheat in Denmark, Finland, Sweden, and Switzerland between 1986 and 1996. In addition to CO<sub>2</sub> enrichment of the air, these experiments included increases and decreases in atmospheric ozone (O<sub>3</sub>). Pleijel *et al.* found when increasing O<sub>3</sub> pollution reduced wheat grain yield, it simultaneously increased the protein concentration of the grain. They also found when O<sub>3</sub> was scrubbed from the air and grain yield was thereby increased, the protein concentration of the grain was decreased. This same relationship described the degree to which grain protein concentration dropped when atmospheric CO<sub>2</sub> enrichment increased grain yield. Hence, whenever the grain yield of the wheat was changed—by CO<sub>2</sub>, O<sub>3</sub>, or even water stress, which was also a variable in one of the experiments—grain protein concentrations moved up or down along a common linear relationship in the opposite direction to the change in grain yield.

In an earlier study of CO<sub>2</sub> and O<sub>3</sub> effects on wheat grain yield and quality, Rudorff *et al.* (1996) obtained essentially the same result. They observed, for example, “flour protein contents were increased by enhanced O<sub>3</sub> exposure and reduced by elevated CO<sub>2</sub>,” but “the combined effect of these gases was minor.” Hence, they conclude, “the concomitant increase of CO<sub>2</sub> and O<sub>3</sub> in the troposphere will have no significant impact on wheat grain quality.”

Evans (1993) found similar relationships for several other crops, further observing them to be greatly affected by soil nitrogen availability. It is highly likely, therefore, that the differing availability of soil nitrogen could have been responsible for some of the results observed in the many other studies reviewed by Idso and Idso (2001). That is precisely what the study of Rogers *et al.* (1996) suggests. Although they observed CO<sub>2</sub>-induced reductions in the protein concentration of flour derived from wheat

plants growing at low soil nitrogen concentrations, no such reductions were evident when the soil nitrogen supply was increased. Hence, Pleijel *et al.* conclude, the oft-observed negative impact of atmospheric CO<sub>2</sub> enrichment on grain protein concentration probably would be alleviated by higher applications of nitrogen fertilizers.

Kimball *et al.* (2001) confirmed that hypothesis. They studied the effects of a 50% increase in atmospheric CO<sub>2</sub> concentration on wheat grain nitrogen concentration and the baking properties of the flour derived from that grain throughout four years of free-air CO<sub>2</sub> enrichment experiments. In the first two years of their study, soil water content was an additional variable, and in the last two years, soil nitrogen content was a variable. The most influential factor in reducing grain nitrogen concentration was determined to be low soil nitrogen. Under this condition, atmospheric CO<sub>2</sub> enrichment further reduced grain nitrogen and protein concentrations, although the change was much less than that caused by low soil nitrogen. When soil nitrogen was not limiting, however, increases in the air's CO<sub>2</sub> concentration did not affect grain nitrogen and protein concentrations, nor did they reduce the baking properties of the flour derived from the grain. Hence, it would appear that given sufficient water and nitrogen, atmospheric CO<sub>2</sub> enrichment can increase wheat grain yield without sacrificing grain protein concentration in the process.

Atmospheric CO<sub>2</sub> enrichment has been found in some studies to increase the protein concentration of wheat. Agrawal and Deepak (2003), for example, grew two cultivars of wheat (*Triticum aestivum* L. cv. Malviya 234 and HP1209) in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 600 ppm alone and in combination with 60 ppb SO<sub>2</sub> to study the interactive effects of elevated CO<sub>2</sub> and this major air pollutant on crop growth. They found exposure to the elevated SO<sub>2</sub> caused a 13% decrease in foliar protein concentrations in both cultivars, but when the plants were concomitantly exposed to an atmospheric CO<sub>2</sub> concentration of 600 ppm, leaf protein levels decreased by only 3% in HP1209 and increased by 4% in Malviya 234.

In the case of rice—which according to Wittwer (1995) was “the basic food for more than half the world's population,” supplying “more dietary energy than any other single food”—Jablonski *et al.* (2002) conducted a wide-ranging review of the scientific literature, finding rice, too, appeared to suffer no reduction in grain nitrogen (protein) concentration in response to atmospheric CO<sub>2</sub> enrichment. They also

found no CO<sub>2</sub>-induced decrease in seed nitrogen concentration in the studies of legumes they reviewed. This finding is significant because, as Wittwer (1995) notes, legumes “are a direct food resource providing 20% of the world's protein for human consumption” and “about two thirds of the world's protein concentrate for livestock feeding.” In addition, Jablonski *et al.* found the biomass of the CO<sub>2</sub>-enriched wheat, rice, and legumes was increased significantly above that of the same crops grown in normal air. Hence, there likely will be a vast increase in the total amount of protein that can be made available to humanity in a future CO<sub>2</sub>-enriched world, both directly via food crops and indirectly via livestock.

Thomas *et al.* (2003) noted “oil and protein comprise ~20 and 40%, respectively, of the dry weight of soybean seed.” This “unique chemical composition,” they write, “has made it one of the most valuable agronomic crops worldwide.” In addition, “the intrinsic value of soybean seed is in its supply of essential fatty acids and amino acids in the oil and protein, respectively.” They report Heagle *et al.* (1998) “observed a positive significant effect of CO<sub>2</sub> enrichment on soybean seed oil and oleic acid concentration.”

Legumes and their responses to atmospheric CO<sub>2</sub> enrichment also figure prominently in studies of mixed forage crops. In a study of nitrogen cycling in grazed pastures on the North Island of New Zealand, for example, Allard *et al.* (2003) found under elevated CO<sub>2</sub>, leaves of the individual species exhibited lower nitrogen concentrations but higher water-soluble carbohydrate (WSC) concentrations. They also note “there was a significantly greater proportion of legume in the diet at elevated CO<sub>2</sub>,” and this “shift in the botanical composition towards a higher proportion of legumes counter-balanced the nitrogen decrease observed at the single species scale, resulting in a nitrogen concentration of the overall diet that was unaffected by elevated CO<sub>2</sub>.” They further report, “changes at the species level and at the sward level appeared to combine additively in relation to WSC.” Hence, they note, “as there was a significant correlation between WSC and digestibility (as previously observed by Dent and Aldrich, 1963 and Humphreys, 1989), there was also an increase in digestibility of the high CO<sub>2</sub> forage.” This result, they write, “matches that found in a Mini-FACE experiment under cutting (Teyssonneyre, 2002; Picon-Cochard *et al.*, 2004),” where “digestibility also increased in response to CO<sub>2</sub> despite reduced crude protein concentration.” These data, plus the strong relation-

ship between soluble sugars (rather than nitrogen) and digestibility, led them to suggest “the widespread response to CO<sub>2</sub> of increased soluble sugars might lead to an increase in forage digestibility.”

Luscher *et al.* (2004) found much the same in their review of the subject, which was based primarily on studies conducted at the Swiss FACE facility that hosted what had by then become the world’s longest continuous atmospheric CO<sub>2</sub> enrichment study of a naturally occurring grassland. There, in response to an approximate two-thirds increase in the atmosphere’s CO<sub>2</sub> concentration, leaf nitrogen (N) concentrations of white clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) were reduced by 7% and 18%, respectively, when they were grown separately in pure stands. As Luscher *et al.* report, “the considerably lower concentration of N under elevated CO<sub>2</sub>, observed for *L. perenne* leaves in pure stands, was found to a much lesser extent for *L. perenne* leaves in the bi-species mixture with *T. repens* (Zanetti *et al.*, 1997; Hartwig *et al.*, 2000).” Furthermore, “under elevated CO<sub>2</sub> the proportion of N-rich *T. repens* (40 mg N g<sup>-1</sup> dry matter) increased in the mixture at the expense of the N-poor *L. perenne* (24 mg N g<sup>-1</sup> dry matter when grown in monoculture),” the end result being “the concentration of N in the harvested biomass of the mixture showed no significant reduction.”

That this phenomenon is likely ubiquitous is suggested by the still more comprehensive review by Campbell *et al.* (2000), who analyzed research conducted between 1994 and 1999 by a worldwide network of 83 scientists associated with the Global Change and Terrestrial Ecosystems (GCTE) Pastures and Rangelands Core Research Project 1 (CRP1). This program had resulted in the publication of more than 165 peer-reviewed scientific journal articles. Campbell *et al.* determined from this massive collection of data the legume content of grass-legume swards typically was increased by approximately 10% in response to a doubling of the air’s CO<sub>2</sub> content.

Luscher *et al.* (2004) state, “the nutritive value of herbage from intensively managed grassland dominated by *L. perenne* and *T. repens* ... is well above the minimum range of the concentration of crude protein necessary for efficient digestion by ruminants (Barney *et al.* 1981).” Hence, they conclude, “a small decrease in the concentration of crude protein in intensively managed forage production systems [which may never occur, as noted above] is not likely to have a negative effect on the nutritive value or on the intake of forage.” In addition, in a CO<sub>2</sub>-enriched world of the future there would be

much more such forage produced per unit of land and water devoted to the enterprise, clearly making the ongoing rise in the air’s CO<sub>2</sub> content a big plus for animal husbandry.

Newman *et al.* (2003) investigated the effects of two levels of nitrogen fertilization and an approximate doubling of the air’s CO<sub>2</sub> content on the growth and chemical composition of tall fescue (*Festuca arundinacea* Schreber cv. KY-31), both when infected and uninfected with a mutualistic fungal endophyte (*Neotyphodium coenophialum* Morgan-Jones and Gams). They found the elevated CO<sub>2</sub> reduced the crude protein content of the forage by an average of 21% in three of the four situations studied: non-endophyte-infected plants in both the low and high nitrogen treatments, and endophyte-infected plants in the high nitrogen treatment. There was no protein reduction for endophyte-infected plants in the low nitrogen treatment.

Newman *et al.* note, “the endophyte is present in many native and naturalized populations and the most widely sown cultivars of *F. arundinacea*,” so the first two situations in which the CO<sub>2</sub>-induced protein reduction occurred (those involving non-endophyte-infected plants) are not typical of the real world. In addition, since the dry-weight biomass yield of the forage was increased by fully 53% under the low nitrogen regime, and since the 10-times-greater high nitrogen regime boosted yields only by an additional 8%, there would appear to be no need to apply extra nitrogen to *F. arundinacea* in a CO<sub>2</sub>-enriched environment.

Consequently, under best management practices in a doubled-CO<sub>2</sub> world of the future, little or no nitrogen would be added to the soil, and there would be little or no reduction in the crude protein content of *F. arundinacea*. But there would be more than 50% more of it produced on the same amount of land.

With respect to the final plant quality (forage digestibility) studied by Newman *et al.*, increasing soil nitrogen lowered *in vitro* neutral detergent fiber digestibility in both ambient and CO<sub>2</sub>-enriched air, and this phenomenon was most pronounced in the elevated CO<sub>2</sub> treatment. Under low nitrogen conditions there again was no decline in plant digestibility. Hence, there is a second good reason not to apply extra nitrogen to *F. arundinacea* in a high CO<sub>2</sub> world of the future, and little need to do so.

With respect to the unmanaged world of nature, increases in the air’s CO<sub>2</sub> content often, but not always (Goverde *et al.*, 1999), lead to greater decreases in the concentrations of nitrogen and protein in the foliage of C<sub>3</sub> as compared to C<sub>4</sub> grasses

(Wand *et al.*, 1999). As a result, in the words of Barbehenn *et al.* (2004a), “it has been predicted that insect herbivores will increase their feeding damage on C<sub>3</sub> plants to a greater extent than on C<sub>4</sub> plants (Lincoln *et al.*, 1984, 1986; Lambers, 1993).”

To test this hypothesis, Barbehenn *et al.* (2004a) grew *Lolium multiflorum* Lam. (Italian ryegrass, a common C<sub>3</sub> pasture grass) and *Bouteloua curtipendula* (Michx.) Torr. (sideoats gramma, a native C<sub>4</sub> rangeland grass) in chambers maintained at either the ambient atmospheric CO<sub>2</sub> concentration of 370 ppm or the doubled CO<sub>2</sub> concentration of 740 ppm for two months, after which newly molted sixth-instar larvae of *Pseudaletia unipuncta* (a grass-specialist noctuid) and *Spodoptera frugiperda* (a generalist noctuid) were allowed to feed on the grasses. As expected, foliage protein concentration decreased by 20% in the C<sub>3</sub> grass, but by only 1% in the C<sub>4</sub> grass, when grown in the CO<sub>2</sub>-enriched air. However, and “contrary to our expectations,” Barbehenn *et al.* write, “neither caterpillar species significantly increased its consumption rate to compensate for the lower concentration of protein in [the] C<sub>3</sub> grass,” and “this result does not support the hypothesis that C<sub>3</sub> plants will be subject to greater rates of herbivory relative to C<sub>4</sub> plants in future [high-CO<sub>2</sub>] atmospheric conditions (Lincoln *et al.*, 1984).” In addition, “despite significant changes in the nutritional quality of *L. multiflorum* under elevated CO<sub>2</sub>,” they report “no effect on the relative growth rate of either caterpillar species on either grass species resulted,” and there were “no significant differences in insect performance between CO<sub>2</sub> levels.”

In a similar study, Barbehenn *et al.* (2004b) allowed grasshopper (*Melanoplus sanguinipes*) nymphs reared to the fourth-instar stage to feed on the grasses. Once again, “contrary to the hypothesis that insect herbivores will increase their feeding rates disproportionately in C<sub>3</sub> plants under elevated atmospheric CO<sub>2</sub>,” they found “*M. sanguinipes* did not significantly increase its consumption rate when feeding on the C<sub>3</sub> grass grown under elevated CO<sub>2</sub>.” They suggest this observation implies “post-ingestive mechanisms enable these grasshoppers to compensate for variable nutritional quality in their host plants,” and some of these post-ingestive responses may include “changes in gut size, food residence time, digestive enzyme levels, and nutrient metabolism (Simpson and Simpson, 1990; Bernays and Simpson, 1990; Hinks *et al.*, 1991; Zanutto *et al.*, 1993; Yang and Joern, 1994a,b).” Their data indicate *M. sanguinipes* growth rates may have increased, perhaps

by as much as 12%, when feeding upon the C<sub>3</sub> foliage that had been produced in the CO<sub>2</sub>-enriched air.

With respect to both managed agricultural crops and the wild plants of Earth’s natural ecosystems, it appears the ongoing rise of the air’s CO<sub>2</sub> concentration will have few negative impacts of any consequence on the nutritive value of their grains and foliage in terms of protein concentration. In tree crops such as citrus, CO<sub>2</sub>-induced changes in the activities of certain foliar proteins could lead to vast increases in their yield potential, as elucidated by the work of Idso *et al.* (2001).

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## 2.22 Respiration

Nearly all of Earth's plants respond favorably to increases in the air's CO<sub>2</sub> concentration by exhibiting enhanced rates of photosynthesis and biomass production during the light part of each day. However, the observed increases in these parameters (especially biomass production) are also due, in part, to CO<sub>2</sub>-induced reductions in carbon losses via respiration during both the day and night (the latter called "dark respiration"). The subsections below examine what has been learned about both of these types of respiration from experiments conducted on various herbaceous and woody plants.

### 2.22.1 Herbaceous Plants

The growth and biomass production of herbaceous crops generally is enhanced by CO<sub>2</sub>-induced decreases in respiration during the dark (nighttime) period. However, fundamental information is still lacking on how respiration and the processes

supported by it are physiologically controlled, thereby preventing sound interpretations of what seem to be species-specific responses of respiration to elevated CO<sub>2</sub>. The precise role of plant respiration in augmenting the sink capacity of herbaceous plants remains uncertain.

#### 2.22.1.1 Crops

- The growth and biomass production of herbaceous crops generally is enhanced by CO<sub>2</sub>-induced decreases in respiration during the dark (nighttime) period.

Baker *et al.* (2000) grew rice in Soil-Plant-Atmosphere Research (SPAR) units at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm during daylight hours. Under these conditions, rates of dark respiration decreased in both CO<sub>2</sub> treatments with short-term increases in the air's CO<sub>2</sub> concentration at night. However, when dark respiration rates were measured at the CO<sub>2</sub> growth concentrations of the plants, they were not significantly different from each other.

Cousins *et al.* (2001) grew sorghum at atmospheric CO<sub>2</sub> concentrations of 370 and 570 ppm in a free-air CO<sub>2</sub> enrichment (FACE) facility near Phoenix, Arizona (USA). Within six days of planting, the photosynthetic rates of the second leaves of the CO<sub>2</sub>-enriched plants were 37% greater than those of the second leaves of the ambiently grown plants. This CO<sub>2</sub>-induced photosynthetic enhancement slowly declined with time, stabilizing at about 15% between 23 and 60 days after planting. In addition, when measuring photosynthetic rates at a reduced oxygen concentration of 2%, they observed 16 and 9% increases in photosynthesis for the ambient and CO<sub>2</sub>-enriched plants, respectively, which suggests the extra 200 ppm of CO<sub>2</sub> was reducing photorespiratory carbon losses, although this phenomenon did not account for all of the CO<sub>2</sub>-induced stimulation of photosynthesis.

Das *et al.* (2002) grew tropical nitrogen-fixing mungbean plants in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 350 or 600 ppm for two growing seasons, with the extra CO<sub>2</sub> being provided between either days 0 and 20 or days 21 and 40 after germination. The elevated CO<sub>2</sub> decreased rates of respiration by 54 to 62%, with the greatest declines occurring during the first 20 days after germination.

Wang *et al.* (2004) grew well-watered and

fertilized South American tobacco plants from seed in 8.4-liter pots (one plant per pot) filled with sand and housed in controlled-environment growth chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 365 or 730 ppm for nine weeks. Over this period they found the ratio of net photosynthesis per unit leaf area (A) to dark respiration per unit leaf area (Rd) “changed dramatically.” Whereas A/Rd was the same in both treatments at the beginning of the measurement period, a month later it had doubled in the CO<sub>2</sub>-enriched environment but had risen by only 58% in the ambient CO<sub>2</sub> treatment. Speaking of this finding, the three researchers state, “if the dynamic relationship between A and Rd observed in *N. sylvestris* is applicable to other species, it will have important implications for carbon cycling in terrestrial ecosystems, since plants will assimilate CO<sub>2</sub> more efficiently as they mature.”

Bunce (2005) grew soybeans in the field in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of ambient and ambient +350 ppm at the Beltsville Agricultural Research Center in Maryland (USA), where net carbon dioxide exchange rate measurements were performed on a total of 16 days between 18 July and 11 September of 2000 and 2003, during the flowering to early pod-filling stages of the growing season. Averaging his results over the course of the study, Bunce found daytime net photosynthesis per unit leaf area was 48% greater in the plants growing in the CO<sub>2</sub>-enriched air, and nighttime respiration per unit leaf area was unaffected by elevated CO<sub>2</sub>. However, because the extra 350 ppm of CO<sub>2</sub> increased leaf dry mass per unit area by an average of 23%, respiration per unit of mass was significantly lower for the leaves of the soybeans growing in the CO<sub>2</sub>-enriched air.

Wang and Curtis (2002) conducted a meta-analysis of the results of 45 area-based dark respiration (Rda) and 44 mass-based dark respiration (Rdm) assessments of the effects of a doubling of the air's CO<sub>2</sub> concentration on 33 species of plants derived from 37 scientific studies. The mean leaf Rda of the suite of herbaceous plants studied was significantly higher (+29%,  $P < 0.01$ ) at elevated CO<sub>2</sub> than at ambient CO<sub>2</sub>. When the herbaceous plants were separated into groups that had experienced durations of CO<sub>2</sub> enrichment either shorter or longer than 60 days, the short-term studies exhibited a mean Rda increase of 51% ( $P < 0.05$ ), and the long-term studies exhibited no effect. Hence, for conditions of continuous atmospheric CO<sub>2</sub> enrichment, herbaceous plants likely would experience no change in leaf Rda. In addition, the two researchers found plants exposed

to elevated CO<sub>2</sub> for < 100 days “showed significantly less of a reduction in leaf Rdm due to CO<sub>2</sub> enrichment (-12%) than did plants exposed for longer periods (-35%,  $P < 0.01$ ).” Hence, for long-term conditions of continuous atmospheric CO<sub>2</sub> enrichment, herbaceous crops likely would experience an approximate 35% decrease in leaf Rdm.

Bunce (2004) grew six 16-plant batches of soybeans in a single controlled-environment chamber, one to a pot filled with 1.8 liters of vermiculite that was flushed daily with a complete nutrient solution. In three experiments conducted at day/night atmospheric CO<sub>2</sub> concentrations of 370/390 ppm, air temperatures were either 20, 25, or 30°C, and in three other experiments conducted at an air temperature of 25°C, atmospheric CO<sub>2</sub> concentrations were either 40, 370, or 1,400 ppm. At the end of the normal 16 hours of light on the 17th day after planting, half of the plants were harvested and used for the measurement of a number of physical parameters, and measurements of the plant physiological processes of respiration, translocation, and nitrate reduction were made on the other half of the plants over the following eight-hour dark period.

Plotting translocation and nitrate reduction as functions of respiration, Bunce found “a given change in the rate of respiration was accompanied by the same change in the rate of translocation or nitrate reduction, regardless of whether the altered respiration was caused by a change in temperature or by a change in atmospheric CO<sub>2</sub> concentration.” Bunce concludes, “the parallel responses of translocation and nitrate reduction for both the temperature and CO<sub>2</sub> treatments make it unlikely that the response of respiration to one variable [CO<sub>2</sub>] was an artifact while the response to the other [temperature] was real.” Hence, there is reason to believe the oft-observed decreases in dark respiration experienced by plants exposed to elevated levels of atmospheric CO<sub>2</sub>, as per the review and analysis studies of Drake *et al.* (1999) and Wang and Curtis (2002), are indeed real and not the result of measurement system defects.

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### 2.22.1.2 Other Herbaceous Plants

- Fundamental information is still lacking on how respiration and the processes supported by it are physiologically controlled in herbaceous plants, thereby preventing sound interpretations of what seem to be species-specific responses of respiration to elevated CO<sub>2</sub>. The role of plant respiration in augmenting the sink capacity of terrestrial ecosystems remains uncertain.

Rabha and Uprety (1998) grew India mustard plants for an entire season in open-top chambers with either ambient or enriched (600 ppm) atmospheric CO<sub>2</sub> concentrations and adequate or inadequate soil moisture levels. The elevated CO<sub>2</sub> concentration reduced leaf dark respiration rates by about 25% in both soil moisture treatments, which suggests a greater proportion of the increased carbohydrate pool

in the CO<sub>2</sub>-enriched plants remained within them to facilitate increases in growth and development.

Ziska and Bunce (1999) grew four C<sub>4</sub> plants in controlled environment chambers maintained at either full-day (24-hour) atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm or a nocturnal-only CO<sub>2</sub> concentration of 700 ppm (with 350 ppm CO<sub>2</sub> during the day) for about three weeks. In this study, 24-hour CO<sub>2</sub> enrichment caused a significant increase in the photosynthesis (+13%) and total dry mass (+21%) of only one of the four C<sub>4</sub> species (*Amaranthus retroflexus*). There was no significant effect of nocturnal-only CO<sub>2</sub> enrichment on this species, indicating the observed increase in biomass, resulting from 24-hour atmospheric CO<sub>2</sub> enrichment, was not facilitated by greater carbon conservation stemming from a CO<sub>2</sub>-induced reduction in dark respiration.

Grunzweig and Korner (2001) constructed model grasslands representative of the Negev of Israel and placed them in growth chambers maintained at atmospheric CO<sub>2</sub> concentrations of 280, 440, and 600 ppm for five months. They also found atmospheric CO<sub>2</sub> enrichment had no effect on nighttime respiratory carbon losses.

Van der Heijden *et al.* (2000) grew peat moss hydroponically within controlled environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm for up to six months, subjecting the peat moss to three different levels of nitrogen deposition. In all cases, they found the elevated CO<sub>2</sub> reduced rates of dark respiration consistently throughout the study by 40 to 60%.

Gonzalez-Meler *et al.* (2004) reviewed the scientific literature on the effects of atmospheric CO<sub>2</sub> enrichment on plant respiration from the cellular level to the level of entire ecosystems. They report finding, “contrary to what was previously thought, specific respiration rates are generally not reduced when plants are grown at elevated CO<sub>2</sub>.” Nevertheless, they note, “whole ecosystem studies show that canopy respiration does not increase proportionally to increases in biomass in response to elevated CO<sub>2</sub>.” This suggests respiration per unit biomass likely is reduced somewhat by atmospheric CO<sub>2</sub> enrichment. However, they also found “a larger proportion of respiration takes place in the root system [when plants are grown in CO<sub>2</sub>-enriched air],” which once again obfuscates the issue.

The three researchers remark, “fundamental information is still lacking on how respiration and the processes supported by it are physiologically controlled, thereby preventing sound interpretations of what seem to be species-specific responses of

respiration to elevated CO<sub>2</sub>,” concluding “the role of plant respiration in augmenting the sink capacity of terrestrial ecosystems is still uncertain.”

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### 2.22.2 Woody Plants

- Both above and below the soil surface, coniferous trees appear to exhibit significant reductions in respiration in CO<sub>2</sub>-enriched air. However, deciduous trees exhibit both increases and decreases, as well as cases of no change. It again appears atmospheric CO<sub>2</sub> enrichment may either increase or decrease woody-plant respiration, but not to any great degree, so the net result for Earth’s trees likely would be of little significance.

Plant growth rates are determined largely by the relative magnitudes of carbon gain via photosynthesis and carbon loss via respiration. Although much is known about the effects of atmospheric CO<sub>2</sub> enrichment on the first of these phenomena, much less is known about its effects on the second. The subsections below examine the various effects of CO<sub>2</sub>-enriched air on respiration in various types of woody plants.

#### 2.22.2.1 Coniferous Trees

- Both above and below the soil surface, coniferous trees may exhibit significant reductions in respiration in a high-CO<sub>2</sub> world of the future.

Jach and Ceulemans (2000) grew three-year old Scots pine seedlings out-of-doors and rooted in the ground in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 350 or 750 ppm for two years. To make the experiment more representative of the natural world, they applied no nutrients or irrigation water to the soils in which the trees grew for the duration of the study. After two years of growth under these conditions, dark respiration on a needle mass basis in the CO<sub>2</sub>-enriched seedlings was 27 and 33% lower in current-year and one-year-old needles, respectively. The researchers conclude the greater reduction in the older needles arises from the greater duration of elevated CO<sub>2</sub> exposure experienced by those needles.

Hamilton *et al.* (2001) studied the short- and long-term respiratory responses of loblolly pines in a free-air CO<sub>2</sub>-enrichment (FACE) study established in 1996 on 13-year-old trees in a North Carolina (USA) plantation, where the CO<sub>2</sub>-enriched trees were exposed to an extra 200 ppm of CO<sub>2</sub>. This modest increase in the atmosphere’s CO<sub>2</sub> concentration produced no significant short-term suppression of dark respiration rates in the trees’ needles. Neither did long-term exposure to elevated CO<sub>2</sub> alter maintenance respiration, the amount of CO<sub>2</sub> that is respired to maintain existing plant tissues. However, growth respiration, the amount of CO<sub>2</sub> respired when constructing new tissues, was reduced by 21%.

McDowell *et al.* (1999) grew five-month-old seedlings of western hemlock in root boxes subjected to various root-space CO<sub>2</sub> concentrations (ranging from 90 to 7,000 ppm) for periods of several hours to determine the effects of soil CO<sub>2</sub> concentration on growth, maintenance, and total root respiration. Although elevated CO<sub>2</sub> had no effect on growth respiration, it significantly affected maintenance and total respiration. At a soil CO<sub>2</sub> concentration of 1585 ppm, for example, total and maintenance respiration rates of roots were 55 and 60% lower, respectively, than at 395 ppm. The impact of elevated CO<sub>2</sub> on maintenance respiration was so strong, in fact, it exhibited an exponential decline of about 37% for every doubling of soil CO<sub>2</sub> concentration. This observation is especially important because maintenance respiration comprised 85% of total root respiration in this study.

George *et al.* (2003) begin their work by noting, “several studies have documented a decrease in the specific rate of fine-root respiration for trees grown in elevated atmospheric CO<sub>2</sub>,” citing Callaway *et al.* (1994), BassiriRad *et al.* (1997), and Crookshanks *et al.* (1998). Then, citing Cotrufo *et al.* (1998) as a basis for the idea that “growth under elevated CO<sub>2</sub> causes a decrease in the nitrogen concentration of roots,” which in turn suggests “a reduction in protein concentration,” they hypothesize, “the energy required for protein turnover may decline in elevated CO<sub>2</sub> causing a reduction in maintenance respiration,” and the resultant “decrease in maintenance respiration with elevated CO<sub>2</sub> may contribute to increases in growth respiration.”

To test this hypothesis, George *et al.* measured the maintenance respiration of non-growing fine roots in the absence of nutrients, while quantifying growth respiration from calculated construction costs and the observed production rates of fine roots in two major forest FACE studies: the Duke Forest study of a loblolly pine (*Pinus taeda* L.) plantation and the Oak Ridge National Laboratory (ORNL) Forest study of a sweetgum (*Liquidambar styraciflua* L.) plantation.

Based on unpublished data of R.J. Norby and D.W. Johnson from the ORNL Forest and the data of Finzi *et al.* (2002) from the Duke Forest, George *et al.* report, contrary to their initial assumption, as well as that of Hungate *et al.* (2003), they “were unable to detect an effect of elevated CO<sub>2</sub> on the nitrogen concentration of fine roots for either species.” They found a significant reduction of fine-root maintenance respiration in the loblolly pine plantation, but no significant difference in this parameter between the two sweetgum CO<sub>2</sub> treatments. Assessments of CO<sub>2</sub>-induced increases in fine-root production in the study of Matamala and Schlesinger (2000) for the loblolly pines and in the study of Norby *et al.* (2002) for the sweetgum trees revealed fine-root biomass increases of 87% in the first case and 77% in the second case in response to an approximate 200-ppm increase in atmospheric CO<sub>2</sub> concentration.

George *et al.* state, “the C:N ratio of fine roots grown in elevated CO<sub>2</sub> was not altered and consequently did not explain the trend of reduced annual fine-root maintenance respiration and the increase in annual fine-root growth respiration for loblolly pine.” Instead, they conclude, “for these forests it appears that an increase in fine-root production is the primary factor contributing to the increase in annual growth respiration under elevated CO<sub>2</sub>.” Hence, they end up rejecting their original hypothesis.

In a non-CO<sub>2</sub>-enrichment study, Bronson and Gower (2010) state, “the boreal forest historically has been considered a carbon sink,” but “autotrophic respiration is [supposedly] more sensitive than photosynthesis to increases in temperature (Ryan, 1991; Amthor, 1994).” Therefore, in response to global warming, “most models predict autotrophic respiration will increase at a greater rate than photosynthesis, which infers decreased carbon use efficiency and net primary production (Ryan, 1995; VEMAP Members, 1995; Ryan *et al.*, 1996).” This further implies “a substantial increase in temperature could turn the boreal forest into a carbon source (Goulden *et al.*, 1998),” and this positive feedback could lead to an intensification of global warming.

Bronson and Gower studied black spruce trees (*Picea mariana* (Mill.) B.S.P.) growing in large, enclosed greenhouse chambers about 20 km south of Thompson, Manitoba, Canada (55°53'N, 98°20'W). Soil-heating cables were used to warm air and soil temperatures about 5°C over ambient control temperatures. The researchers measured light-saturated net photosynthesis, foliage respiration, and stem respiration in heated and control forest plots during the 2005, 2006, and 2007 growing seasons. Throughout the study, they found “both the older foliage, which developed before the experiment, and the new foliage, developed during the experiment, had similar rates of light-saturated net photosynthesis, foliage respiration and stem respiration across all treatments,” which, they write, “underscores the ability of black spruce to maintain homeostasis in a 5°C warmer environment.” In addition, while noting many global change models predict a doubling of respiration for every 10°C increase in temperature, Bronson and Gower state in the concluding sentence of their paper, “the results from this and other whole-ecosystem warming experiments do not support this model assumption.”

Lhotakova *et al.* (2012) measured rates of light-saturated net photosynthesis and dark respiration in current-year needles of sun-exposed (3rd whorl) and shaded (6th whorl) parts of the canopies of originally 10-year-old Norway spruce (*Picea abies*) trees that were transplanted into native soil (which was given an initial fertilizer treatment) and grown for an additional eight years in the Beskydy Mountains of the Czech Republic within semi-open glass domes having adjustable windows, where the trees were exposed to either the ambient-air CO<sub>2</sub> concentration (AC, which varied from 365 to 377 ppm over the course of the long-term experiment) or to an enriched-air CO<sub>2</sub> concentration (EC, which was

maintained at a steady value of 700 ppm).

Based on the graphical representations of Lhotakova *et al.*'s findings, it can be calculated that at the end of the eight-year CO<sub>2</sub> enrichment experiment there was a CO<sub>2</sub>-induced increase in light-saturated net photosynthesis of approximately 115% in the trees' Sun-exposed needles and about 55% in their shaded needles. It can likewise be calculated there was an approximate 20% decrease in the mean dark respiration rate of the trees' Sun-exposed needles and about 40% in their shaded needles.

The nine Czech scientists conclude, "the positive effect of EC on net CO<sub>2</sub> assimilation rates, as we observed in juvenile Norway spruce trees in the present study, was also shown in other long-term studies on conifers," citing Maier *et al.* (2008), Kosvancova *et al.* (2009), Crous *et al.* (2008), and Logan *et al.* (2009). In addition, "it appears that forest trees, including conifers, suppress respiration rates under long-term CO<sub>2</sub> enrichment," citing Zhou *et al.* (2007) and Gonzalez-Meler *et al.* (2009). Thus they conclude, "the observed stimulation of light-saturated net photosynthesis simultaneously with suppressed dark respiration under EC may lead to higher biomass accumulation," as Runion *et al.* (2006) had observed earlier and reported for longleaf pine trees.

The results of the experiments described in this section suggest coniferous trees may exhibit reductions in respiration both above and below the soil surface in a high-CO<sub>2</sub> world of the future.

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### 2.22.2.2 Deciduous Trees

- Several experiments conducted on deciduous trees indicate there are both increases and decreases in respiration rates in response to atmospheric CO<sub>2</sub> enrichment, as well as cases of no change in respiration. More data are needed before any general conclusions may be drawn.

Wang and Curtis (2001) grew cuttings of two male and two female trembling aspen trees for about five months on soils containing low and high nitrogen contents in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 380 and 765 ppm. They found gender had little effect on dark respiration rates, but elevated CO<sub>2</sub> increased them, by 6% and 32% in the low and high soil nitrogen treatments, respectively. On the other hand, Karnosky *et al.* (1999) grew both O<sub>3</sub>-sensitive and O<sub>3</sub>-tolerant aspen clones for one full year in free-air CO<sub>2</sub>-enrichment (FACE) plots maintained at atmospheric CO<sub>2</sub> concentrations of 360 and 560 ppm, finding the extra CO<sub>2</sub> decreased dark respiration rates by 24%.

Gielen *et al.* (2003) measured stem respiration rates of white, black, and robusta poplar trees in a high-density forest plantation in the third year of a FACE experiment in which the CO<sub>2</sub> concentration of the air surrounding the trees was increased to a value of approximately 550 ppm. They write, “stem respiration rates were not affected by the FACE treatment,” and “FACE did not influence the relationships between respiration rate and both stem temperature and relative growth rate.” In addition, they found “no effect of the FACE treatment on R<sub>m</sub> [maintenance respiration, which is related to the sustaining of existing cells] and R<sub>g</sub> [growth respiration, which is related to the synthesis of new tissues].”

Hamilton *et al.* (2001) studied respiratory responses of sweetgum trees growing in the understory of a loblolly pine plantation (but occasionally reaching the top of the canopy) to an extra 200 ppm of CO<sub>2</sub> in a FACE study conducted in North Carolina (USA). They determined the modest increase in the air's CO<sub>2</sub> concentration did not appear to alter maintenance respiration to any significant degree, but it reduced dark respiration by an average of 10% and growth respiration of leaves at the top of the canopy by nearly 40%.

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### 2.22.2.3 Multiple Tree Studies

- Experimental results are contradictory, suggesting atmospheric CO<sub>2</sub> enrichment may either increase, decrease, or have little effect on woody-plant respiration.

Amthor (2000) measured dark respiration rates of intact leaves of nine tree species growing naturally in an American deciduous forest. Within a specially designed leaf chamber, the CO<sub>2</sub> concentration surrounding individual leaves was stabilized at 400 ppm for 15 minutes, and their respiration rates were measured for 30 minutes, after which the CO<sub>2</sub> concentration in the leaf chamber was raised to 800 ppm for 15 minutes and respiration data were again recorded for the same leaves. This revealed elevated CO<sub>2</sub> had little effect on leaf dark respiration rates. The extra 400 ppm of CO<sub>2</sub> within the measurement cuvette decreased the median respiration rate by only 1.5% across the nine tree species; this observation led Amthor to state the “rising atmospheric CO<sub>2</sub> concentration has only a small direct effect on tree leaf respiration in deciduous forests.” He calculated that effect could be “more than eliminated by a 0.22°C temperature increase.” He thus concludes, “future direct effects of increasing CO<sub>2</sub> in combination with warming could stimulate tree leaf respiration in their sum,” and this consequence “would translate into only slight, if any, effects on the carbon balance of temperate deciduous forests in a future atmosphere containing as much as [800 ppm] CO<sub>2</sub>.”

Amthor’s conclusion is debatable, however, because it is based on extrapolation of the short-term respiratory responses of individual leaves exposed to elevated CO<sub>2</sub> for only an hour or two, to that of entire trees, many of which will experience rising CO<sub>2</sub> levels for a century or more during their lifetimes. Trees are long-lived organisms that should not be expected to reveal the nature of their long-term responses to elevated atmospheric CO<sub>2</sub> concentrations on as short a time scale as 15 minutes. Their respiratory responses may change significantly with the passage of time as they acclimate and optimize their physiology and growth patterns to the gradually rising CO<sub>2</sub> content of Earth’s atmosphere, as evidenced by the findings of two other studies.

In the first study, Wang and Curtis (2002) conducted a meta-analysis of the results of 45 area-based dark respiration (R<sub>da</sub>) and 44 mass-based dark respiration (R<sub>dm</sub>) assessments of the effects of an approximate doubling of the air’s CO<sub>2</sub> concentration on 33 species of plants (both herbaceous and woody) derived from 37 scientific publications. The mean leaf R<sub>da</sub> of the woody plants they analyzed was unaffected by elevated CO<sub>2</sub>. There was, however, an effect on mean leaf R<sub>dm</sub>, and it was determined to be time-dependent. The woody plants exposed to elevated CO<sub>2</sub> for less than 100 days, the reviewing scientists write, “showed significantly less of a reduction in leaf R<sub>dm</sub> due to CO<sub>2</sub> enrichment (-12%) than did plants exposed for longer periods (-35%, P < 0.01).” Hence, for conditions of continuous long-term atmospheric CO<sub>2</sub> enrichment, the results of Wang and Curtis’s analysis suggest woody plants may experience an approximate 35% decrease in leaf R<sub>dm</sub>.

In the second study, Drake *et al.* (1999) also conducted a comprehensive analysis of the peer-reviewed scientific literature to determine the effects of elevated atmospheric CO<sub>2</sub> concentrations on plant respiration rates. They found atmospheric CO<sub>2</sub> enrichment typically decreased respiration rates in mature foliage, stems, and roots of CO<sub>2</sub>-enriched plants relative to rates measured in plants grown in ambient air. When normalized on a biomass basis, they determined a doubling of the atmosphere’s CO<sub>2</sub> concentration likely would reduce plant respiration rates by an average of 18%. To determine the potential effects of this phenomenon on annual global carbon cycling, which the 12 researchers say “will enhance the quantity of carbon stored by forests,” they put a 15% CO<sub>2</sub>-induced respiration reduction into a carbon sequestration model, finding an additional 6 to 7 Gt of carbon would remain seques-

tered within the terrestrial biosphere each year, substantially strengthening the terrestrial carbon sink.

Davey *et al.* (2004) reached a different conclusion, noting, “Averaged across many previous investigations, doubling the CO<sub>2</sub> concentration has frequently been reported to cause an instantaneous reduction of leaf dark respiration measured as CO<sub>2</sub> efflux.” But they point out “no known mechanism accounts for this effect, and four recent studies [Amthor (2000); Amthor *et al.* (2001); Jahnke (2001); Jahnke and Krewitt (2002)] have shown that the measurement of respiratory CO<sub>2</sub> efflux is prone to experimental artifacts that could account for the reported response.”

Davey *et al.* employed a high-resolution dual channel oxygen analyzer in an open gas exchange system to measure the respiratory O<sub>2</sub> uptake of nine species of plants in response to a short-term increase in atmospheric CO<sub>2</sub> concentration, as well as the response of seven species to long-term elevation of the air’s CO<sub>2</sub> content in four field experiments. They found “[more than] six hundred separate measurements of respiration failed to reveal any decrease in respiratory O<sub>2</sub> uptake with an instantaneous increase in CO<sub>2</sub>.” Nor could they detect any response to a fivefold increase in the air’s CO<sub>2</sub> concentration or the total removal of CO<sub>2</sub> from the air. They also note, “this lack of response of respiration to elevated CO<sub>2</sub> was independent of treatment method, developmental stage, beginning or end of night, and the CO<sub>2</sub> concentration at which the plants had been grown.” In the long-term field studies, there was a respiratory response, but it was small (7% on a leaf mass basis), and it was positive, not negative.

The most reasonable conclusion from these contradictory results is that atmospheric CO<sub>2</sub> enrichment induces both increases and decreases in respiration rates, as well as cases of no change in respiration. More data are needed before any general conclusions may be drawn.

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## 2.23 Roots

As indicated in the subsections below, elevated CO<sub>2</sub> nearly always increases root biomass—often even more than shoot biomass—by increasing the size of taproots and the number and size of lateral roots, along with fine-root biomass and a number of other important root properties.

### 2.23.1 Crops

- As the air’s CO<sub>2</sub> content continues to rise, most crops will develop larger and more extensive root systems that should help them to better cope with periods of reduced soil moisture. This chain of events should make the soil environment more favorable for plant growth and development in a high-CO<sub>2</sub> world of the future.

Hodge and Millard (1998) grew narrowleaf plantain (*Plantago lanceolata*) seedlings for six weeks in controlled environment growth rooms maintained at atmospheric CO<sub>2</sub> concentrations of either 400 or 800 ppm. By the end of this period, the plants in the 800-ppm air exhibited increases in shoot and root dry matter production 159 and 180% greater, respectively, than the corresponding dry matter increases experienced by the plants growing in 400-ppm air, and the amount of plant carbon recovered from the potting medium (sand) was 3.2 times greater in the

elevated-CO<sub>2</sub> treatment. Thus, the belowground growth stimulation provided by atmospheric CO<sub>2</sub> enrichment was greater than that experienced aboveground.

Wechsung *et al.* (1999) grew spring wheat (*Triticum aestivum*) in rows in a FACE study employing atmospheric CO<sub>2</sub> concentrations of 370 and 550 ppm and irrigation treatments that periodically replaced either 50 or 100% of prior potential evapotranspiration, in an effort to determine the effects of elevated CO<sub>2</sub> and water stress on root growth. They found elevated CO<sub>2</sub> increased in-row root dry weight by an average of 22% during the growing season under both the wet and dry irrigation regimes. In addition, during the vegetative growth phase, atmospheric CO<sub>2</sub> enrichment increased inter-row root dry weight by 70%, indicating plants grown in elevated CO<sub>2</sub> developed greater lateral root systems than plants grown at ambient CO<sub>2</sub>. During the reproductive growth phase, elevated CO<sub>2</sub> stimulated the branching of lateral roots into inter-row areas, but only when water was limiting to growth. In addition, the CO<sub>2</sub>-enriched plants tended to display greater root dry weights at a given depth than did ambiently grown plants.

In a comprehensive review of all prior FACE experiments conducted on agricultural crops, Kimball *et al.* (2002) determined for a 300-ppm increase in atmospheric CO<sub>2</sub> concentration, the root biomass of wheat, ryegrass, and rice experienced an average increase of 70% at ample water and nitrogen, 58% at low nitrogen, and 34% at low water. Clover experienced a 38% increase at ample water and nitrogen and a 32% increase at low nitrogen. Cotton exhibited a 96% increase in root biomass at ample water and nitrogen.

Zhao *et al.* (2000) germinated pea (*Pisum sativum*) seeds and exposed the young plants to various atmospheric CO<sub>2</sub> concentrations in controlled environment chambers to determine whether elevated CO<sub>2</sub> affects root border cells, which are major contributors of root exudates in this and most other agronomic plants. They found elevated CO<sub>2</sub> increased the production of root border cells in pea seedlings. In going from ambient air to air enriched to 3,000 and 6,000 ppm CO<sub>2</sub>, border-cell numbers increased by more than 50 and 100%, respectively. Hence, as the CO<sub>2</sub> content of the air continues to rise, peas (and possibly many other crop plants) likely will produce greater numbers of root border cells, which should increase the amounts of root exudations occurring in their rhizospheres, which further suggests associated soil microbial and fungal activities will be stimulated

as a result of the increases in plant-derived carbon inputs these organisms require to meet their energy needs.

Van Ginkel *et al.* (1996) grew perennial ryegrass (*Lolium perenne*) plants from seed in two growth chambers for 71 days under continuous <sup>14</sup>CO<sub>2</sub>-labeling of the atmosphere at CO<sub>2</sub> concentrations of 350 and 700 ppm at two soil nitrogen levels. At the conclusion of this part of the experiment, the plants were harvested and their roots dried, pulverized, and mixed with soil in one-liter pots placed in two wind tunnels in an open field, one of which had ambient air of 361 ppm CO<sub>2</sub> flowing through it, and one of which had air of 706 ppm CO<sub>2</sub> flowing through it. Several of the containers were then seeded with more *Lolium perenne*, others were similarly seeded the following year, and still others were kept bare for two years. At the ends of the first and second years, the different degrees of decomposition of the original plant roots were assessed.

The scientists determined shoot and root growth were enhanced by 13 and 92%, respectively, by the extra CO<sub>2</sub> in the initial 71-day portion of the experiment, again demonstrating the significant benefits often conferred on plant roots by atmospheric CO<sub>2</sub> enrichment. They also found the decomposition of the high-CO<sub>2</sub>-grown roots in the high-CO<sub>2</sub> wind tunnel was 19% lower than that of the low-CO<sub>2</sub>-grown roots in the low-CO<sub>2</sub> wind tunnel at the end of the first year. Decomposition was 14% lower at the end of the second year in the low-nitrogen-grown plants but equivalent in the high-nitrogen-grown plants. It was also determined the presence of living roots reduced the decomposition rate of dead roots below the dead-root-only decomposition rate observed in the bare soil treatment. Van Ginkel *et al.* conclude, “the combination of higher root yields at elevated CO<sub>2</sub> combined with a decrease in root decomposition will lead to a longer residence time of C in the soil and probably to a higher C storage.”

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### 2.23.2 Grasses

- Root biomass in grasslands may have increased markedly as the air's CO<sub>2</sub> content rose following the termination of the last glacial period, but even more substantial increases are expected if the air's CO<sub>2</sub> content continues to rise as projected, implying ever-greater grassland root biomass.

Many and varied experiments have addressed how the roots of grasses respond to increases in the air's CO<sub>2</sub> concentration and/or soil temperature. Regarding warming, Fitter *et al.* (1999) studied a site on the Great Dun Fell in the United Kingdom, where they determined root birth and death rates from biweekly minirhizotron video images obtained over one experiment of six months' duration and another of 18 months' duration in an upland grassland, where the soils of half of the treatment replications were artificially maintained 2.8°C above ambient at a depth of 2 cm. This warming increased both root production and root death by approximately equivalent amounts. Therefore, they conclude, "the effect of a warmer climate will be a similar acceleration in both [root] birth and death processes and these will have no direct effect on the soil carbon store."

Regarding atmospheric CO<sub>2</sub> enrichment, Crookshanks *et al.* (1998) sprouted seeds of the small and fast-growing *Arabidopsis thaliana* plant on agar medium in Petri dishes and grew the resulting immature plants in controlled-environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 355 or 700 ppm. Visual assessments of root growth were made after emergence of the roots from the seeds, and microscopic investigations of root cell properties were conducted. The scientists found the CO<sub>2</sub>-enriched plants directed a greater proportion of their newly produced biomass into root- as opposed to shoot-growth, and the young plants produced longer

primary roots and more and longer lateral roots. These effects were found to be related to the CO<sub>2</sub>-induced stimulation of mitotic activity, accelerated cortical cell expansion, and increased cell wall plasticity.

Milchunas *et al.* (2005) conducted a five-year open-top chamber study (ambient CO<sub>2</sub> = 360 ppm, enriched CO<sub>2</sub> = 720 ppm) in semiarid shortgrass steppe grassland at the USDA-ARS Central Plains Experimental Range in north central Colorado (USA), where 88% of the ecosystem's biomass was provided by three codominant species—*Bouteloua gracilis* (H.B.K.) Lag., *Stipa comata* (Trin and Rupr.), and *Pascopyrum smithii* (Rybd.)—and where a subfrutescent shrub (*Artemisia frigida* Willd.) was also abundant. They obtained video-image data on root growth and decay in each treatment-replicate from 18 minirhizotron tubes that acquired video images to a depth of 40 cm four to five times a year. Root biomass data were obtained from two 20-cm-diameter cylinders driven into the ground within each chamber and collected yearly in the autumn.

They found root-length growth was 52% greater in the CO<sub>2</sub>-enriched chambers than in the ambient-air chambers, and root-length losses were 37% greater in the elevated-CO<sub>2</sub> air. The difference between the CO<sub>2</sub>-induced growth and decay stimulations was largely attributable to the 41% longer lifespan of the CO<sub>2</sub>-enriched roots, which resulted in a CO<sub>2</sub>-induced root-length pool size increase of 41%. In the upper part of the soil profile, root diameters typically were observed to be larger in the CO<sub>2</sub>-enriched chambers, leading to an ultimate CO<sub>2</sub>-induced root biomass increase of 59%. The four researchers conclude the "slower turnover of new soil carbon, and increased life span of roots suggest an increased storage of carbon under elevated CO<sub>2</sub>."

Ayres *et al.* (2008) report the responses of belowground nematode herbivores to atmospheric CO<sub>2</sub> enrichment to approximately 350 ppm above ambient in experiments conducted on three grassland ecosystems in Colorado and California (USA) and Montpellier, France. They found soil moisture increased in response to elevated CO<sub>2</sub> in all three experiments, citing Hungate *et al.* (1997), Nijs *et al.* (2000), and Morgan *et al.* (2004), and "elevated CO<sub>2</sub> increased root biomass by approximately 3–32% in the first five years of the Coloradoan study (Pendall *et al.*, 2004), by 23% after six years in the Californian study (Rillig *et al.*, 1999), and by 31% after six months in the French study (Dhillon *et al.*, 1996)." With respect to the nematodes, they state, "CO<sub>2</sub> enrichment did not significantly affect the family

richness, diversity, or plant parasitic nematode index of herbivorous nematodes in the Colorado, California, or French study,” noting “in each experiment, neutral effects were the most frequent response to CO<sub>2</sub> enrichment.” The seven researchers conclude, “one consequence of increased root production, without changes in belowground herbivore populations, might be greater plant inputs to soil,” which “may lead to greater soil organic matter pools in grassland ecosystems, potentially enhancing soil carbon sequestration.”

Adair *et al.* (2009) employed mass balance calculations to quantify the effects of biodiversity, atmospheric CO<sub>2</sub> concentration, and soil nitrogen (N) content on the total amount of carbon (C) allocated belowground by plants (total belowground C allocation or TBCA), and ecosystem C storage, in an eight-year experiment that was part of the BioCON study of a periodically burned Minnesota grassland. They found annual TBCA increased in response to all three treatment variables—elevated CO<sub>2</sub>, enriched N, and increasing diversity—and it was also “positively related to standing root biomass.” Upon removing the influence of root biomass, however, the effects of N and diversity became neutral or even negative (depending on the year), but “the effect of elevated CO<sub>2</sub> remained positive.” In years with fire, they found “greater litter production in high diversity, elevated CO<sub>2</sub>, and enhanced N treatments increased annual ecosystem C loss.” Therefore, under non-fire conditions, elevated CO<sub>2</sub>, N, and biodiversity generally would tend to increase ecosystem carbon gain, but if grasslands are frequently burned, they could remain neutral in this regard.

Anderson *et al.* (2010) studied various root responses of a C<sub>3</sub>-C<sub>4</sub> grassland community at Temple, Texas (USA) over a CO<sub>2</sub> concentration gradient stretching from 230 to 550 ppm, which they created in two CO<sub>2</sub>-gradient above-ground “tunnels” of clear polyethylene film. One of the 60-m-long and 1.5-m-wide chambers had ambient air pumped into one end of it; by the time that air exited the chamber through its other end, its CO<sub>2</sub> concentration was reduced by the photosynthetic activity of the plants within the chamber to a value of approximately 230 ppm. The other chamber had air enriched to a CO<sub>2</sub> concentration of 550 ppm pumped into one end of it; as this air exited out the other end of that chamber, its CO<sub>2</sub> concentration was reduced to a value approximately equivalent to that of the ambient air (~380 ppm). The researchers assessed community ingrowth root biomass along the lengths of the tunnels every two to four months from May 1997 through

November 1999, using two ingrowth cores in each five-meter chamber section, and they calculated root biomass response as the ratio of each measurement date’s result to that prevailing at the start of the experiment in May 1997.

Based on the linear relationship they derived from the 20 ingrowth biomass assessments they conducted, Anderson *et al.* found “a 40% increase in the ingrowth root biomass ratio from 380 to 480 ppm as compared with a 36% increase from 280 to 380 ppm.” When excluding one extremely variable data point and using a power function they fit to the data, “the contrast is even greater: a 50% increase from 380 to 480 ppm vs. a 41% increase from 280 to 380 ppm.” In going from the linear relationship to the power function, the *r*<sup>2</sup> value of the relationship jumped from 0.10 to 0.50, and *P* dropped from 0.095 to less than 0.001.

The six scientists conclude, “root biomass in grasslands may have changed markedly as atmospheric CO<sub>2</sub> increased since the last glacial period, but ... more substantial changes are ahead if the air’s CO<sub>2</sub> content doubles by the end of this century as predicted.” Those anticipated “changes” should all be positive, implying greater grassland root biomass—and all the good things that phenomenon implies—as the air’s CO<sub>2</sub> content continues to climb.

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### 2.23.3 Trees

The ongoing rise in the air's CO<sub>2</sub> content enables Earth's conifers and deciduous trees to increase the volume of soil from which they access water and nutrients, thus allowing them to more thoroughly explore that enlarged volume of soil and acquire greater amounts of these essential resources needed for growth. Numerous experimental findings indicate possible concurrent increases in air temperature and nitrogen deposition will enable them to become even more robust and productive.

#### 2.23.3.1 Conifers

- The ongoing rise in the air's CO<sub>2</sub> content enables Earth's conifers to increase the volume of soil from which they access water and nutrients, allowing them to more thoroughly explore that enlarged volume of soil and acquire more of these essential resources for growth, the aerial fertilization effect of atmospheric CO<sub>2</sub> enrichment.

Janssens *et al.* (1998) grew three-year-old Scots pine seedlings for six months in open-top chambers maintained at ambient and 700 ppm atmospheric CO<sub>2</sub> concentrations. They found the extra CO<sub>2</sub> increased total root length by 122% and total root dry mass by 135%. In a study that employed close to the same degree of enhancement of the atmosphere's CO<sub>2</sub> content, Pritchard *et al.* (2001a) grew ecosystems representative of regenerating longleaf pine forests of the southeastern USA for 18 months in large soil bins located within open-top chambers. They found the aboveground parts of the seedlings experienced a growth enhancement of only 20%, but the root biomass of the trees was increased by more than three times as much (62%).

Working with FACE technology, Pritchard *et al.* (2001b) studied 14-year-old loblolly pine trees after a year of exposure to an extra 200 ppm of CO<sub>2</sub>. They found total root length and root numbers were 16 and 34% greater, respectively, in the CO<sub>2</sub>-enriched plots than in the ambient-air plots. In addition, the elevated CO<sub>2</sub> increased the diameter of living and dead roots by 8 and 6%, respectively, and annual root production was 26% greater in the CO<sub>2</sub>-enriched plots. For the degree of CO<sub>2</sub> enrichment used in the prior two studies, this latter increase corresponded to a root biomass increase of about 45%.

In an open-top chamber study of a model ecosystem composed of a mixture of spruce and beech seedlings, Wiemken *et al.* (2001) investigated the effects of a 200-ppm increase in the air's CO<sub>2</sub> concentration that prevailed for four years. On nutrient-poor soils, the extra CO<sub>2</sub> led to a 30% increase in fine-root biomass, and on nutrient-rich soils it led to a 75% increase. As before, these numbers corresponded to increases of about 52% and 130%, respectively, for atmospheric CO<sub>2</sub> enhancements on the order of those employed by Janssens *et al.* (1998) and Pritchard *et al.* (2001a).

Wiemken *et al.* also found the extra CO<sub>2</sub> increased the amount of symbiotic fungal biomass associated with the trees' fine roots by 31% on nutrient-poor soils and 100% on nutrient-rich soils, which for the degree of atmospheric CO<sub>2</sub> enrichment used in the studies of Janssens *et al.* (1998) and Pritchard *et al.* (2001a) translate into increases of about 52% and 175%, respectively.

Tingey *et al.* (2005) used minirhizotron tubes to study the effects of atmospheric CO<sub>2</sub> enrichment (to approximately 350 ppm above ambient) on the fine-root architecture of seedlings of Ponderosa pine (*Pinus ponderosa*) growing in open-top chambers at the U.S. Forest Service's Institute of Forest Genetics

near Placerville, California (USA), over a period of four years. They found elevated CO<sub>2</sub> increased both fine root extensity (the degree of soil exploration) and intensity (the extent that roots use explored areas) but had no effect on mycorrhizae. They presume the latter observation to be due to the fact that soil nitrogen was not limiting to growth in their study. More specifically, they report, “extensity increased 1.5- to 2-fold in elevated CO<sub>2</sub> while intensity increased only 20% or less,” noting similar extensity results were obtained over shorter periods of four months to two years by Arnon (1997), Berntson and Bazzaz (1998), DeLucia *et al.* (1997), and Runion *et al.* (1997), and similar intensity results had been obtained by Berntson (1994).

Phillips *et al.* (2006) grew well-watered (via drip irrigation) 1.5-year-old ponderosa pine seedlings for four additional years in open-top chambers maintained at the ambient atmospheric CO<sub>2</sub> concentration and at ambient + 175 ppm and ambient + 300 ppm CO<sub>2</sub>, while imposing three levels of soil nitrogen (N) fertilization (0, 10 and 20 g N m<sup>-2</sup> year<sup>-1</sup>) upon the plants. Every two months throughout this period, they collected video images of the roots that were visible on the surfaces of three minirhizotron tubes installed in each chamber. They learned yearly values of fine-root standing crop, production, and mortality were consistently higher in the elevated CO<sub>2</sub> treatments throughout the study. They also report, “in this same study, Johnson *et al.* (2000) found elevated CO<sub>2</sub> increased fine-root life span,” and because the elevated CO<sub>2</sub> increased fine-root length, the amount of root length that died each year was greater in the CO<sub>2</sub> enriched treatments. Therefore, they write, “the higher rates of mortality in absolute terms for elevated CO<sub>2</sub> are driven by increased standing crop and not reduced life spans.”

The work of Phillips *et al.* came to bear upon the progressive nitrogen limitation (PNL) hypothesis, which posits the benefits of atmospheric CO<sub>2</sub> enrichment must ultimately dwindle away because the productivity of Earth's temperate forests often is limited by insufficient soil nitrogen, as is typically the case in the southeastern United States, where the growth of pine and hardwood forests can remove so much nitrogen from the soils on which the forests grow that it induces what Finzi and Schlesinger (2003) have described as “a state of acute nutrient deficiency that can only be reversed with fertilization.” Phillips *et al.* found, however, the increased fine-root length they observed “explains how additional N was provided to support the increased whole plant growth observed in [their]

elevated CO<sub>2</sub> treatments, and corresponds with the increased extent and intensity of the root system architecture discussed by Tingey *et al.* (2005).” This “mining of soil N,” as they describe it, “can in some cases go on for substantial lengths of time,” and there was “no evidence that PNL occurred during the course of [their] study,” citing Johnson *et al.* (2006).

Pritchard *et al.* (2008) described what they learned from minirhizotrons they employed in work conducted at the Duke Forest FACE facility to characterize the influence of free-air-CO<sub>2</sub>-enrichment (ambient + 200 ppm) on fine roots for six years (Autumn 1998 through Autumn 2004) in an 18-year-old loblolly pine (*Pinus taeda*) plantation near Durham, North Carolina (USA). Over six years they observed the extra 200 ppm of CO<sub>2</sub> increased average fine-root standing crop by 23%, in line with the overall stimulation of forest net primary productivity of 18–24% they observed for 1996–2002. They found “the positive effects of CO<sub>2</sub> enrichment on fine root growth persisted six years following minirhizotron tube installation (eight years following initiation of the CO<sub>2</sub> fumigation),” and no hint of any progressive nitrogen limitation of the stimulatory effect of atmospheric CO<sub>2</sub> enrichment in a situation where one might have been expected it. In partial explanation of this important finding, Pritchard *et al.* conclude the distal tips of fine roots are “the primary site for initiation of mycorrhizal partnerships which are critical for resource acquisition and could also influence whether or not forests can sustain higher productivity in a CO<sub>2</sub>-enriched world.”

Phillips *et al.* (2009) write, “O<sub>3</sub> stress often decreases carbon allocation to roots, leading to reductions in root biomass and growth,” citing Andersen (2003) and Grantz *et al.* (2006). They add, “reduced carbohydrate stores in roots can lead to increased susceptibility to other stresses even after O<sub>3</sub> exposure ends,” citing Andersen *et al.* (1997), but stating, on the other hand, CO<sub>2</sub> tends to promote just the opposite behavior by promoting fine-root production and the benefits this phenomenon provides, citing Norby *et al.* (2004, 2005). In an experiment designed to determine which of the two trace gases (CO<sub>2</sub> or O<sub>3</sub>) has the greater impact on the growth and development of the fine roots of ponderosa pine trees, they grew *Pinus ponderosa* seedlings for three years in one-meter-deep containers filled with reconstructed pine-forest soil within sunlit controlled-environment chambers maintained at mean atmospheric CO<sub>2</sub> concentrations of either 420 or 690 ppm, and at mean O<sub>3</sub> conditions described by daily SUM06 index values of either 0 or 15.7 ppm h

(representing the sum of hourly O<sub>3</sub> concentrations  $\geq$  0.06 ppm). Images of fine roots growing along the upper surfaces of four minirhizotron tubes installed within each soil bin were collected every 28 days by a color video camera.

The researchers found “elevated CO<sub>2</sub> increased both the number of fine roots produced and their life span,” and “increased O<sub>3</sub> did not reduce the effect of elevated CO<sub>2</sub>.” Fine root biomass at the end of the study in the CO<sub>2</sub>-enriched treatment was consistently higher in each soil horizon and 16% higher in total. In addition, the greater fine-root survivorship in the elevated CO<sub>2</sub> treatment was associated with increasing root depth and increasing fine-root diameter, as also has been observed by Eissenstat *et al.* (2000), Guo *et al.* (2008), and Joslin *et al.* (2006). Averaged over the course of the experiment, they found a slight (3.3%) decrease in soil respiration in the elevated CO<sub>2</sub> treatment, as observed by Tingey *et al.* (2006). Consequently, in the words of the four U.S. Environmental Protection Agency scientists who conducted the work, “elevated O<sub>3</sub> did not result in significant negative impacts on ponderosa pine seedling fine-root survival ... or in countering the increased survivorship caused by elevated CO<sub>2</sub>.”

Johansson *et al.* (2009) write, “ectomycorrhizal (ECM) fungi, forming the dominant type of symbiotic association with trees in boreal forests, receive as much as 25% of the total carbon assimilated by plants,” and in return, “the extraradical fungal mycelium is directly involved in mobilization and uptake of nutrients which are, in part, passed on to the host plant.” This important function is performed by the fungal exudation of a variety of low molecular weight organic compounds, polymer degrading enzymes, siderophores, polymeric carbohydrates, and fatty acids, the dominant components of which “play important roles in enhancing mineral weathering, nutrient mobilization and uptake by plants.” They investigated certain aspects of this complex suite of phenomena in seedlings of Scots pine (*Pinus sylvestris*) trees grown in the laboratory in liquid culture for six weeks with either no ECM fungi or one of eight such species associated with their roots, during which period they were exposed to air of either 350 or 700 ppm CO<sub>2</sub>, and after which a number of analyses were performed to identify and quantify the variety of exudates produced by the fungi.

Johansson *et al.* observed “a clear impact of elevated CO<sub>2</sub> on exudation of soluble low molecular weight organic compounds,” and these exudates “increased by 120–270%” due to “the increased carbon availability to the plant-fungus system.” This

process was driven by the elevated atmospheric CO<sub>2</sub> concentration that increased net CO<sub>2</sub> assimilation rates by approximately 40% for both ECM and non-mycorrhizal seedlings. It led to a mean increase of 27% in the total biomass production of the seedlings infected with the eight species of ECM fungi, but it led to only a 14% increase in the biomass of the non-infected seedlings. Therefore, the four researchers conclude the phenomena they observed “may contribute to nutritional feedback mechanisms to sustain tree growth when nutrients become limiting,” such as some have hypothesized might occur over time in trees growing on low-fertility soils in CO<sub>2</sub>-enriched air. (For much more on this subject, see the Progressive Limitation Hypothesis, Section 3.11, this volume.) The findings of this study, however, as well as those of the study of de Graaff *et al.* (2009)—which was published in the same issue of *Soil Biology & Biochemistry*—clearly indicate Earth’s plants should be well-equipped to deal with this hypothetical and now largely discredited roadblock to higher plant productivity in a CO<sub>2</sub>-enriched world of the future.

The results described above add to the growing body of evidence suggesting the ongoing rise in the atmosphere’s CO<sub>2</sub> concentration will enable conifers to continue to increase the volume of soil from which they can access water and nutrients, enabling them to more thoroughly explore that enlarged volume of soil. Both of these responses should allow them to acquire more of these essential resources and thereby realize the enhanced potential for growth that is provided them by the aerial fertilization effect of atmospheric CO<sub>2</sub> enrichment. The ongoing rise in the air’s CO<sub>2</sub> content thus bodes well for the growth of the planet’s coniferous forests and for all the creatures that depend upon them for food and shelter, as well as for the ability of the trees to provide lumber for mankind and to sequester carbon while doing so. These consequences have great virtue in and of themselves, and the latter one provides a powerful negative feedback or brake on CO<sub>2</sub>-induced global warming.

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### 2.23.3.2 Deciduous

- Experimental findings indicate the ongoing rise in the air's CO<sub>2</sub> content, together with possible concurrent increases in air temperature and nitrogen deposition, will make Earth's deciduous woody plants increasingly robust and productive.

Although life-sustaining photosynthesis occurs aboveground in the presence of light, what happens in the darkness beneath the surface of the soil is equally important to the welfare of Earth's higher plants and the many benefits they provide for the rest of the biosphere. This section highlights the findings of some of the scientific literature germane to this subject, focusing on root responses of deciduous trees to atmospheric CO<sub>2</sub> enrichment.

Berntsen and Bazzaz (1998) removed intact chunks of soil from the Hardwood-White Pine-Hemlock forest region of New England and placed them in plastic containers within controlled environment glasshouses maintained at either 375 or 700 ppm CO<sub>2</sub> for two years to study the effects of elevated CO<sub>2</sub> on the regeneration of plants from seeds and rhizomes in the soil. At the conclusion of the study, total mesocosm plant biomass (more than 95% of which was supplied by yellow and white birch tree seedlings) was 31% higher in the elevated CO<sub>2</sub> treatment than in ambient air, with a mean enhancement of 23% aboveground and 62% belowground. The extra CO<sub>2</sub> also increased the mycorrhizal colonization of root tips by 45% in white birch and 71% in yellow birch, and the CO<sub>2</sub>-enriched yellow birch seedlings exhibited 322% greater root length and 305% more root surface area than did the yellow birch seedlings growing in ambient air.

Kubiske *et al.* (1998) grew cuttings of four quaking aspen genotypes in open-top chambers for five months at atmospheric CO<sub>2</sub> concentrations of either 380 or 720 ppm and low or high soil nitrogen concentrations. The cuttings grown in elevated CO<sub>2</sub> displayed no discernible increases in aboveground

growth. However, the extra CO<sub>2</sub> significantly increased fine-root length and root turnover rates at high soil nitrogen by increasing fine-root production, which would be expected to produce benefits, not the least of which would be a larger belowground water- and nutrient-gathering system, that would eventually lead to enhanced aboveground growth as well. The many positive aboveground growth responses of quaking aspen trees to atmospheric CO<sub>2</sub> enrichment are documented in Appendices 2 and 3 of this volume (see *Populus tremuloides* Michx.).

Expanding on this study, Pregitzer *et al.* (2000) grew six quaking aspen genotypes for 2.5 growing seasons in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm with both adequate and inadequate supplies of soil nitrogen. The trees exposed to elevated CO<sub>2</sub> developed thicker and longer roots than the trees growing in ambient air, and the fine-root biomass of the CO<sub>2</sub>-enriched trees was enhanced by 17% in the nitrogen-poor soils and by 65% in the nitrogen-rich soils.

Another study of quaking aspen, conducted by King *et al.* (2001), demonstrates trees exposed to an atmospheric CO<sub>2</sub> concentration 560 ppm in a FACE experiment produced 133% more fine-root biomass than trees grown in ambient air of 360 ppm, which roughly equates to 233% more fine-root biomass for the degree of CO<sub>2</sub> enrichment employed in the prior study of Pregitzer *et al.* where the CO<sub>2</sub> concentration was 700 ppm. When simultaneously exposed to air of 1.5 times the normal ozone concentration, the degree of fine-root biomass stimulation produced by the extra CO<sub>2</sub> was still as great as 66%, or roughly 115% when extrapolated to the greater CO<sub>2</sub> enrichment employed by Pregitzer *et al.*

King *et al.* (1999) grew four clones at two temperature regimes (separated by 5°C) and two levels of soil nitrogen (N) availability (high and low) for 98 days, measuring photosynthesis, growth, biomass allocation, and root production and mortality. They found the higher of the two temperature regimes increased rates of photosynthesis by 65% and rates of whole-plant growth by 37%, simultaneously enhancing root production and turnover. They conclude, "trembling aspen has the potential for substantially greater growth and root turnover under conditions of warmer soil at sites of both high and low N-availability" and "an immediate consequence of this will be greater inputs of C and nutrients to forest soils."

These several findings pertaining to quaking aspen trees indicate increases in atmospheric CO<sub>2</sub> concentration, air temperature, and soil nitrogen

content all enhance the trees' belowground growth, which positively affects their aboveground growth.

Regarding other deciduous trees, Gleadow *et al.* (1998) grew eucalyptus seedlings for six months in glasshouses maintained at atmospheric CO<sub>2</sub> concentrations of either 400 or 800 ppm, fertilizing them twice daily with low or high nitrogen solutions. The elevated CO<sub>2</sub> increased total plant biomass by 98 and 134% relative to plants grown at ambient CO<sub>2</sub> in the high and low nitrogen treatments, respectively. In addition, in the low nitrogen treatment, elevated CO<sub>2</sub> stimulated greater root growth, as indicated by a 33% higher root:shoot ratio.

Day *et al.* (1996) studied the effects of elevated CO<sub>2</sub> on fine-root production in open-top chambers erected over a regenerating oak-palmetto scrub ecosystem in Florida (USA). They found a 350-ppm increase in the atmosphere's CO<sub>2</sub> concentration increased fine-root length densities by 63% while enhancing the distribution of fine roots at the soil surface (0–12 cm) and at a depth of 50–60 cm. These findings suggest the ongoing rise in the atmosphere's CO<sub>2</sub> concentration likely will increase the distribution of fine roots near the soil surface, where the greatest concentrations of nutrients are located, and at a depth that coincides with the upper level of the site's water table, both of which should increase the trees' ability to acquire the nutrients and water they will need to support CO<sub>2</sub>-enhanced biomass production in the future.

Uselman *et al.* (2000) grew seedlings of the nitrogen-fixing black locust tree for 100 days in controlled environments maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm and air temperatures of 26°C (ambient) and 30°C, with either some or no additional nitrogen fertilization. The extra CO<sub>2</sub> increased total seedling biomass by 14%, the elevated temperature increased it by 55%, and nitrogen fertilization increased it by 157%. Root exudation showed a similar pattern. Plants grown in elevated CO<sub>2</sub> exuded 20% more organic carbon compounds than plants grown in ambient air, and elevated temperature and fertilization increased root exudation by 71 and 55%, respectively. Hence, as the air's CO<sub>2</sub> content continues to rise, black locust trees likely will exhibit enhanced rates of biomass production and exudation of dissolved organic compounds from their roots. Moreover, if air temperature also rises, even by as much as 4°C, its positive effect on biomass production and root exudation likely will be even greater than that resulting from the increasing atmospheric CO<sub>2</sub> concentration. The same would appear to hold true for

anthropogenic nitrogen deposition, reinforcing what was learned about the impacts of these three environmental factors on the growth of quaking aspen trees.

McDowell *et al.* (1999) grew five-month-old seedlings of western hemlock in root boxes, subjecting them for several hours to various root-space CO<sub>2</sub> concentrations, ranging from approximately 90 to 7,000 ppm, to determine the effect of soil CO<sub>2</sub> concentration on growth, maintenance, and total root respiration. Although they could detect no effect of atmospheric CO<sub>2</sub> enrichment on growth respiration, it significantly affected maintenance and total respiration rates. At a soil CO<sub>2</sub> concentration of 1,585 ppm, for example, total and maintenance respiration rates were 55 and 60% lower, respectively, than they were at a soil CO<sub>2</sub> concentration of 395 ppm. The impact of elevated soil CO<sub>2</sub> on maintenance respiration (which comprised 85% of the total respiration in this study) was so strong it exhibited an exponential decline of about 37% for every doubling of the soil CO<sub>2</sub> concentration.

These several experimental findings allow confidence in the conclusion that the ongoing rise in the air's CO<sub>2</sub> content, together with possible concurrent increases in air temperature and nitrogen deposition, likely will make Earth's woody plants increasingly robust and productive.

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#### 2.23.4 Other

In reviewing the scientific literature pertaining to atmospheric CO<sub>2</sub> enrichment effects on belowground plant growth and development, Weihong *et al.* (2000) briefly summarize what was known about this subject at the turn of the century. They report atmospheric CO<sub>2</sub> enrichment typically enhances the growth rates of roots, especially those of fine roots, and CO<sub>2</sub>-induced increases in root production eventually lead to increased carbon inputs to soils, due to enhanced root turnover and exudation of various organic carbon compounds, which can lead to greater soil carbon sequestration. In addition, they note, increased soil carbon inputs stimulate the growth and activities of soil microorganisms that utilize plant-derived carbon as their primary energy source, and they report subsequently enhanced activities of fungal and bacterial plant symbionts often lead to increased plant nutrient acquisition.

In a more narrowly focused study, Gouk *et al.* (1999) grew specimens of an orchid plantlet, Mokara Yellow, in plastic bags flushed with 350 and 10,000 ppm CO<sub>2</sub> for three months, in order to study the effects of elevated CO<sub>2</sub> on this epiphytic CAM species. The super-elevated CO<sub>2</sub> of their experiment enhanced the total dry weight of the orchid plantlets more than twofold, while increasing the growth of existing roots and stimulating the induction of new roots from internodes located on the orchid stems. Total chlorophyll content also was increased in CO<sub>2</sub>-enriched air, by 64 percent in young leaves and by 118 percent in young roots. This phenomenon permit-

ted greater light harvesting during photosynthesis and likely led to the tissue starch contents of the CO<sub>2</sub>-enriched plantlets rising nearly 20-fold higher than those of the control-plantlets. In spite of this large CO<sub>2</sub>-induced accumulation of starch, however, no damage or disruption of chloroplasts was evident in the leaves and roots of the CO<sub>2</sub>-enriched plants.

A final question that has periodically intrigued researchers is whether plants take up carbon through their roots in addition to through their leaves. Although a definitive answer remains elusive, Idso (1989) described various aspects of the issue a quarter-century ago:

“Although several investigators have claimed that plants should receive little direct benefit from dissolved CO<sub>2</sub> (Stolwijk *et al.*, 1957; Skok *et al.*, 1962; Splittstoesser, 1966), a number of experiments have produced significant increases in root growth (Erickson, 1946; Leonard and Pinckard, 1946; Geisler, 1963; Yorgalevitch and Janes, 1988), as well as yield itself (Kursanov *et al.*, 1951; Grinfeld, 1954; Nakayama and Bucks, 1980; Baron and Gorski, 1986), with CO<sub>2</sub>-enriched irrigation water. Early on, Misra (1951) suggested that this beneficent effect may be related to CO<sub>2</sub>-induced changes in soil nutrient availability; and this hypothesis may well be correct. Arteca *et al.* (1979), for example, have observed K, Ca and Mg to be better absorbed by potato roots when the concentration of CO<sub>2</sub> in the soil solution is increased; while Mauney and Hendrix (1988) found Zn and Mn to be better absorbed by cotton under such conditions, and Yurgalevitch and Janes (1988) found an enhancement of the absorption of Rb by tomato roots. In all cases, large increases in either total plant growth or yield accompanied the enhanced uptake of nutrients. Consequently, as it has been suggested that CO<sub>2</sub> concentration plays a major role in determining the porosity, plasticity and charge of cell membranes (Jackson and Coleman, 1959; Mitz, 1979), which could thereby alter ion uptake and organic acid production (Yorgalevitch and Janes, 1988), it is possible that some such suite of mechanisms may well be responsible for the plant productivity increases often observed to result from enhanced concentrations of CO<sub>2</sub> in the soil solution.”

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## 2.24 Rubisco

Rubisco is the primary carboxylating enzyme used by C<sub>3</sub> plants during photosynthesis to incorporate CO<sub>2</sub> into sugars needed for growth and development. Even C<sub>4</sub> and CAM plants, which use PEP-carboxylase as their primary carboxylating enzyme, utilize rubisco during subsequent secondary CO<sub>2</sub> assimilation events. Thus rubisco is universally present in all the planet's vegetation and is, in fact, the most abundant plant enzyme on the face of the Earth, comprising 40 to 50% of total foliage protein and representing an enormous sink for nitrogen and other valuable resources within plants.

However, rubisco is a bifunctional enzyme that also possesses oxygenation activity, and when oxygenation reactions occur, photorespiration is enhanced, resulting in an increased loss of carbon from plant tissues. Thus, CO<sub>2</sub> and O<sub>2</sub> compete for active sites on rubisco in order to drive photosynthesis and photorespiration, respectively. The following subsections explore how these biochemical processes are affected by the rising CO<sub>2</sub> content of the atmosphere, as well as the implications of these changes for the content and/or activity of rubisco.

### 2.24.1 Agricultural Species

- There is a reduced need for nitrogen investment in leaf rubisco in agricultural crops growing in CO<sub>2</sub>-enriched air because under such conditions plants typically reallocate some of their “surplus” nitrogen to other processes essential for optimal growth and development, without compromising enhanced carbon gains via photosynthesis.

Voluminous experimental data demonstrate atmospheric CO<sub>2</sub> enrichment favors the process of carboxylation over that of oxygenation, which thereby increases photosynthetic rates while decreasing photorespiratory rates. Thus the rising CO<sub>2</sub> content of the air invariably leads to greater rates of net

photosynthesis and a more efficient process of carbon fixation, requiring less rubisco to obtain the carbon needed for plant growth and development under CO<sub>2</sub>-enriched conditions.

As a consequence, plants grown in elevated CO<sub>2</sub> environments often, but not always (Farage *et al.*, 1998), exhibit a certain degree of photosynthetic acclimation or down-regulation, which is typically characterized by reduced amounts of rubisco (Sims *et al.*, 1998; Theobald *et al.*, 1998) and/or decreases in its activation state (Pritchard *et al.*, 2000; Reid *et al.*, 1998). However, in nearly every reported case of CO<sub>2</sub>-induced photosynthetic acclimation, net photosynthetic rates displayed by CO<sub>2</sub>-enriched plants have been significantly greater than those exhibited by plants growing at ambient CO<sub>2</sub> concentrations. This section briefly reviews the photosynthetic acclimation of rubisco within agricultural species subjected to elevated CO<sub>2</sub> concentrations.

Sicher and Bunce (1999) grew potato plants at atmospheric CO<sub>2</sub> concentrations of 350, 530, and 700 ppm over a three-year period, documenting 13 and 21% CO<sub>2</sub>-induced reductions in rubisco concentrations at 530 and 700 ppm CO<sub>2</sub>, respectively. Nevertheless, the rates of photosynthesis in the CO<sub>2</sub>-enriched plants were still 28 and 49% greater than those observed in control plants grown in ambient air. Similarly, Maroco *et al.* (1999) report a tripling of the ambient air's CO<sub>2</sub> concentration increased photosynthetic rates in maize (a C<sub>4</sub> plant) by about 15%, in spite of foliar reductions in both rubisco and PEP-carboxylase concentrations. And Theobald *et al.* (1998) grew spring wheat at twice-ambient CO<sub>2</sub> concentrations, discovering the elevated CO<sub>2</sub> reduced the amount of rubisco required to sustain enhanced rates of photosynthesis, which consequently led to a significant increase in plant nitrogen-use efficiency.

Interestingly, when elevated CO<sub>2</sub> induces photosynthetic acclimation, the phenomenon generally does not occur in every leaf of the plant. Osborne *et al.* (1998), for example, grew wheat plants with an additional 200 ppm of CO<sub>2</sub> and reported CO<sub>2</sub>-induced reductions in foliar rubisco concentrations occurred in a depth-dependent manner, with the reductions increasing with depth in the canopy. Sims *et al.* (1999) observed similar canopy-depth-dependent reductions in the rubisco content of sunflowers. Thus, because CO<sub>2</sub>-induced reductions in rubisco typically occur within only a portion of a plant's total leaf area, most plants still exhibit biomass increases in response to elevated CO<sub>2</sub> exposure in spite of acclimation.

CO<sub>2</sub>-induced photosynthetic acclimation also often results from insufficient plant sink strength,

which can lead to carbohydrate accumulation in source leaves and the subsequent triggering of photosynthetic end-product feedback inhibition that reduces foliar rubisco concentrations. Gesch *et al.* (1998), for example, report rice plants—which have a relatively limited potential for developing additional carbon sinks—grown at an atmospheric CO<sub>2</sub> concentration of 700 ppm exhibited increased leaf carbohydrate contents, which likely reduced *rbcS* mRNA levels and ultimately leaf rubisco protein contents.

In another experiment, Gesch *et al.* (2000) took rice plants growing in ambient air and placed them in an atmospheric CO<sub>2</sub> concentration of 175 ppm, which reduced their photosynthesis rates by 45%. After five days of exposure to this sub-ambient CO<sub>2</sub> concentration, however, the plants manifested an up-regulation of rubisco, which stimulated photosynthetic rates by 35%. Thus, plant acclimation responses can involve both increases and decreases in specific enzymes, depending on the nature of the change in atmospheric CO<sub>2</sub> concentration.

These observations demonstrate the reduced need for nitrogen investment in leaf rubisco in plants growing in CO<sub>2</sub>-enriched environments. Under such conditions, plants are able to reallocate some of their “surplus” nitrogen to other processes essential to optimal growth and development without compromising enhanced carbon gains via photosynthesis. The end result, as almost always observed in well-run experiments, is increased biomass production in CO<sub>2</sub>-enriched air (see Section 1.1.1, this volume).

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### 2.24.2 Grassland Species

- The reduced need for nitrogen investment in leaf rubisco in grassland plants growing in CO<sub>2</sub>-enriched environments enables them to reallocate some of this “surplus” nitrogen to other limiting processes required for optimal growth and development without compromising enhanced carbon gains via photosynthesis.

As is the case with agricultural plants, voluminous experimental data demonstrate atmospheric CO<sub>2</sub> enrichment also favors carboxylation over oxygenation in grassland species, which in turn increases their photosynthetic rates and decreases their photorespiratory rates. That means the rising CO<sub>2</sub> content of the air invariably leads to greater rates of net photosynthesis and a more efficient process of carbon fixation, where once again less rubisco is needed to obtain the carbon required for maximum

plant growth and development under CO<sub>2</sub>-enriched conditions.

Thus, plants grown in elevated atmospheric CO<sub>2</sub> environments often, but not always (Ziska *et al.*, 1999), exhibit some degree of photosynthetic acclimation or down-regulation, typically characterized by reduced amounts of rubisco and/or decreases in its activation state. However, in nearly every reported case of CO<sub>2</sub>-induced photosynthetic acclimation, net photosynthetic rates displayed by CO<sub>2</sub>-enriched plants were still significantly greater than those exhibited by plants growing in ambient air.

Cheng *et al.* (1998), for example, grew the herbaceous plant *Arabidopsis thaliana* at an atmospheric CO<sub>2</sub> concentration of 1,000 ppm for 40 days; they found the elevated CO<sub>2</sub> reduced foliar rubisco contents by 34%. However, the elevated CO<sub>2</sub> enhanced foliar contents of glucose and fructose more than twofold, and starch concentrations were increased more than 3.5-fold. Thus, although elevated CO<sub>2</sub> reduced the amount of rubisco in leaves, photosynthetically derived sugars and starch still accumulated.

Midgley *et al.* (1999) grew four *Leucadendron* species from South Africa in air of twice-ambient CO<sub>2</sub> concentrations, observing a 30% reduction in the activity of rubisco. However, rates of net photosynthesis in the CO<sub>2</sub>-enriched plants were still about 40% greater than rates measured in ambiently grown plants. Bryant *et al.* (1998) reported similar results for chalk grassland species exposed to an atmospheric CO<sub>2</sub> concentration of 600 ppm for 14 months. They note elevated CO<sub>2</sub> caused an average reduction of 32% in the rubisco activity of two forbs and one C<sub>3</sub> grass, while still maintaining photosynthetic rates about 28% greater than those observed in ambiently growing plants. Likewise, after growing three grasslands species from the United Kingdom for two full years at 700 ppm CO<sub>2</sub>, Davey *et al.* (1999) reported elevated CO<sub>2</sub> reduced rubisco activity by an average of 27% and increased photosynthetic rates from 12 to 74% in a nutrient-dependent manner.

In the illuminating study of Rogers *et al.* (1998), swards of perennial ryegrass grown in air containing an extra 240 ppm of CO<sub>2</sub> did not exhibit any reductions in rubisco content as long as they were supplied with high levels of soil nitrogen. In contrast, at low soil nitrogen contents the CO<sub>2</sub>-enriched plants displayed a 25% reduction in rubisco levels prior to mechanical cutting. After cutting, however, which removed a large portion of the plants' leaf area, the CO<sub>2</sub>-enriched plants in low nitrogen completely reversed their acclimation response and increased

their levels of rubisco to facilitate greater carbon uptake to repair the damage.

The bottom-line message of these observations is that the reduced need for nitrogen investment in leaf rubisco in plants growing in CO<sub>2</sub>-enriched environments enables them to reallocate some of this “surplus” nitrogen to other limiting processes required for optimal growth and development—without compromising enhanced carbon gains via photosynthesis.

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- reallocate some of the “surplus” nitrogen they might possess to other processes required for optimal growth and development without compromising enhanced carbon gains via photosynthesis.
- Experimental data typically demonstrate atmospheric CO<sub>2</sub> enrichment favors carboxylation over oxygenation in rubisco, which increases photosynthetic rates while reducing photorespiratory rates. Thus the rising CO<sub>2</sub> content of the air usually leads to greater rates of net photosynthesis and more efficient carbon fixation, such that less rubisco is needed to obtain the carbon required for plant growth and development under CO<sub>2</sub>-enriched conditions.
- As a consequence, trees grown in elevated CO<sub>2</sub> environments often, but not always (Stylinski *et al.*, 2000; Beerling *et al.*, 1998), exhibit some degree of photosynthetic acclimation or down-regulation, which typically is characterized by reduced amounts of rubisco (Gleadow *et al.*, 1998) and/or decreases in its activation state (Hamerlynck *et al.*, 2002; Kubiske *et al.*, 2002). In nearly every reported case of CO<sub>2</sub>-induced photosynthetic acclimation, however, the net photosynthetic rates displayed by CO<sub>2</sub>-enriched plants have been significantly greater than those exhibited by plants growing in ambient-air CO<sub>2</sub> concentrations (Murray *et al.*, 2000).
- Tjoelker *et al.* (1998), for example, found a 210-ppm increase in the air’s CO<sub>2</sub> content increased the average rate of net photosynthesis in aspen and birch seedlings by 57% even while inducing a 24% reduction in foliar rubisco content. Similarly, Takeuchi *et al.* (2001) report aspen seedlings grown at an atmospheric CO<sub>2</sub> concentration of 560 ppm exhibited photosynthetic rates in upper-canopy leaves 26% greater than those displayed by upper-canopy leaves of control plants grown in ambient air, in spite of a 28% decrease in foliar rubisco concentrations. CO<sub>2</sub>-induced reductions in foliar rubisco contents that did not offset CO<sub>2</sub>-induced photosynthetic enhancements also have been reported in oak (Blaschke *et al.*, 2001) and pine (Turnbull *et al.*, 1998) trees.
- In another manifestation of photosynthetic acclimation, Centritto *et al.* (1999) observe elevated CO<sub>2</sub> reduced the activity of rubisco without negating CO<sub>2</sub>-induced increases in photosynthesis, and Centritto and Jarvis (1999) report twice-ambient atmospheric CO<sub>2</sub> concentrations reduced rubisco activity in Sitka spruce needles by 36% while enhancing photosynthetic rates by 62%. Similarly, Turnbull *et al.*, (1998) report pine seedlings grown at 650 ppm CO<sub>2</sub> displayed 40% reductions in rubisco

### 2.24.3 Tree Species

- Woody species growing in CO<sub>2</sub>-enriched environments have a reduced need for nitrogen investment in rubisco, which enables them to

activity while exhibiting photosynthetic rates 31% greater than those observed in ambiently grown control seedlings. Rey and Jarvis (1998) also documented this phenomenon in young silver birch trees.

These observations demonstrate the reduced need for nitrogen investment in rubisco in woody species growing in CO<sub>2</sub>-enriched environments, which enables them to reallocate some of the resultant “surplus” nitrogen they might possess to other processes required for optimal growth and development without compromising enhanced carbon gains via photosynthesis.

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## 2.25 Seeds

Atmospheric CO<sub>2</sub> enrichment has been shown to increase amount, size, weight, carbohydrate content, lipid content, protein content, earlier production, earlier germination, and greater percent germination of various plant seeds, all of which bode well for the future of Earth’s biosphere.

### 2.25.1 Crops

- Rising atmospheric CO<sub>2</sub> concentrations will confer several benefits on the seeds of crop plants, including an increase in seed quantity and improvements in seed quality.

When dealing with agricultural commodities such as grain crops, seeds comprise the yield, and in such cases the biomass of one (seeds) is the biomass of the other (yield). Therefore, when looking for effects of elevated CO<sub>2</sub> on the seeds of such crops, there is a natural interest in something more than just their final

biomass. A number of pertinent papers are explored in this section that consider the sources of biomass production as well as seed properties that go beyond biomass.

In a greenhouse study of the various components of seed biomass production, Palta and Ludwig (2000) grew narrow-leaved lupine in pots filled with soil within Mylar-film tunnels maintained at either 355 or 700 ppm CO<sub>2</sub>. They found the extra CO<sub>2</sub> increased the final number of pods and the number of pods that filled large seeds, while it reduced to zero the number of pods that had small seeds, reduced the number of pods with unfilled seeds from 16 to 1 pod per plant, and increased pod set and dry matter accumulation on the developing branches. These CO<sub>2</sub>-induced improvements in key physiological processes resulted in 47 to 56% increases in dry matter per plant, which led to increases of 44 to 66% in seed yield per plant.

Sanhewe *et al.* (1996) grew winter wheat in polyethylene tunnels maintained at atmospheric CO<sub>2</sub> concentrations of 380 and 680 ppm from the time of seed germination to the time of plant maturity, maintaining a temperature gradient of approximately 4°C in each tunnel. In addition to the elevated CO<sub>2</sub> increasing seed yield per unit area, they found it also increased seed weight, but not seed survival or germination. Increasing air temperature, on the other hand, increased seed longevity across the entire range of temperatures investigated (14 to 19°C).

Thomas *et al.* (2003) grew soybean plants to maturity in sunlit controlled-environment chambers under sinusoidally varying day/night-max/min temperatures of 28/18, 32/22, 36/26, 40/30, and 44/34°C and two levels of atmospheric CO<sub>2</sub> concentration (350 and 700 ppm). The effect of temperature on seed composition and gene expression was “pronounced,” but “there was no effect of CO<sub>2</sub>.” However, they note, “Heagle *et al.* (1998) observed a positive significant effect of CO<sub>2</sub> enrichment on soybean seed oil and oleic acid concentration,” and Thomas *et al.* did find the latter parameters increased with rising temperature, from 28/18 to 44/34°C. In addition, they found, “32/22°C is optimum for producing the highest oil concentration in soybean seed,” “the degree of fatty acid saturation in soybean oil was significantly increased by increasing temperature,” and crude protein concentration increased with temperature to 40/30°C.

Thomas *et al.* note “the intrinsic value of soybean seed is in its supply of essential fatty acids and amino acids in the oil and protein, respectively.” Thus the temperature-driven changes they identified in these parameters, as well as the CO<sub>2</sub> effect observed by

Heagle *et al.*, bode well for the future production of this important crop and its value to society in a CO<sub>2</sub>-enriched and warming world. They do note, however, “temperatures during the soybean-growing season in the southern USA are at, or slightly higher than, 32/22°C,” and warming could negatively impact the soybean oil industry in this region. For the world as a whole, however, warming would be a positive development for soybean production, and in the southern United States, shifts in planting zones could accommodate changing weather patterns associated with this phenomenon.

Ziska *et al.* (2001) grew one modern and eight ancestral soybean genotypes in glasshouses maintained at atmospheric CO<sub>2</sub> concentrations of 400 and 710 ppm, finding the extra CO<sub>2</sub> increased photosynthetic rates by an average of 75%. This enhancement in photosynthetic sugar production led to increases in seed yield that averaged 40% for all cultivars, except for one ancestral variety that exhibited an 80% increase in seed yield. Hence, if plant breeders utilize the highly CO<sub>2</sub>-responsive ancestral cultivar identified in this study in their breeding programs, soybean seed yields could rise even faster and higher in the future.

Fiscus *et al.* (2007) grew well-watered and well-fertilized soybean (*Glycine max* (L.) Merr. cv. Essex) plants from seed to maturity for one full growing season out-of-doors near Raleigh, North Carolina (USA) within open-top chambers, either rooted in the ground or in 21-liter pots (one plant per pot) at equal plant densities per unit ground area, while exposing the plants to charcoal-filtered air maintained at CO<sub>2</sub> concentrations of either 370 or 700 ppm.

Although seed yields in the container-grown plants were about 17% less than those of the plants rooted directly in the ground, the CO<sub>2</sub>-induced enhancement ratios of both sets of plants were not significantly different from each other, averaging approximately 20%. In addition, the six researchers state, “there was a small (3–4%) but highly significant increase in the seed oil concentration due to elevated CO<sub>2</sub>,” and this increase was experienced in both rooting environments. Fiscus *et al.* note, “the 3 to 4% increase in oil per seed would amount to a very substantial increase in oil production on a regional, national, or international scale.” For the year 2005, for example, they calculated “an increase of 3.5% of seed oil concentration could result in an additional 2.9 Tg of seed oil in a future climate with CO<sub>2</sub> concentrations well above current ambient levels.”

Derner *et al.* (2004) determined above- and below-ground responses of three generations of two

genotypes of spring wheat to atmospheric CO<sub>2</sub> enrichment to 336 ppm above ambient. This experiment was conducted in glasshouse bays, where the second- and third-generation plants were progeny of seeds produced by plants grown at either ambient or enriched atmospheric CO<sub>2</sub> concentrations under well-watered and high soil-nutrient conditions.

Neither genotype in the first generation exhibited enhanced growth in response to the increased concentration of atmospheric CO<sub>2</sub>. However, Derner *et al.* report, “relative enhancement occurred in both the second and third generations for both above- and below-ground variables,” and the “relative enhancement of measured variables was generally greater in the third than second generation when plants were in the seedling or vegetative stage.” They also determined “intergenerational above- and below-ground responses of this C<sub>3</sub> annual plant to CO<sub>2</sub> enrichment are not driven by genetic change (selection) that occurred between generations, but rather CO<sub>2</sub>-induced changes in seeds that affected seedling responses to CO<sub>2</sub> enrichment.”

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### 2.25.2 Grasslands

- Atmospheric CO<sub>2</sub> enrichment typically increases the amount, size, and weight of seeds produced and dispersed each year by various grassland species, as well as their carbon/nitrogen ratios and germination percentage. Concomitant increases in air temperature may result in advancements of flowering and fruiting phenology, which can increase spread rates of plant populations because wind conditions in spring tend to produce higher spread rates than wind conditions later in the year.

Steinger *et al.* (2000) collected seeds from *Bromus erectus* plants grown at atmospheric CO<sub>2</sub> concentrations of 360 and 650 ppm, and then germinated some of both groups of seeds under those same two sets of conditions. In the first part of their study, they found the elevated CO<sub>2</sub> treatment increased individual seed mass by about 9% and increased the seed carbon-to-nitrogen ratio by almost 10%. These changes in seed properties had little impact on subsequent seedling growth; when the seeds produced by ambient or CO<sub>2</sub>-enriched plants were germinated and grown in ambient air, there was no significant size difference between the two groups of resultant seedlings after a period of 19 days. Likewise, when the seeds produced from the ambient and CO<sub>2</sub>-enriched plants were germinated and grown in the high CO<sub>2</sub> treatment, there was no significant difference between the sizes of the seedlings derived from the two groups of seeds. However, the CO<sub>2</sub>-enriched seedlings produced from both groups of seeds were almost 20% larger than the seedlings produced from both groups of seeds grown in ambient air, demonstrating the direct effects of atmospheric CO<sub>2</sub> enrichment on seedling growth and development were more important than the differences in seed characteristics produced by the elevated atmospheric CO<sub>2</sub> concentration in which their parent plants grew.

Edwards *et al.* (2001) employed a FACE experiment where daytime atmospheric CO<sub>2</sub> concentrations above a sheep-grazed pasture in New Zealand were increased by 115 ppm, in order to study the effects of elevated CO<sub>2</sub> on seed production, seedling recruitment, and species compositional changes. In the two years of their study, the extra

daytime CO<sub>2</sub> increased seed production and dispersal in seven of the eight most abundant species, including the grasses *Anthoxanthum odoratum*, *Lolium perenne*, and *Poa pratensis*, the legumes *Trifolium repens* and *T. subterranean*, and the herbs *Hypochaeris radicata* and *Leontodon saxatilis*. In some of these plants, elevated CO<sub>2</sub> also increased the number of seeds per reproductive structure, and all exhibited CO<sub>2</sub>-induced increases in the number of reproductive structures per unit of ground area. In addition, the CO<sub>2</sub>-induced increases in seed production contributed to the increase in the numbers of species found within the CO<sub>2</sub>-enriched plots.

In a five-year study of a nutrient-poor calcareous grassland in Switzerland, Thurig *et al.* (2003) used screen-aided CO<sub>2</sub> control (SACC) technology (Leadley *et al.*, 1997) to enrich the air over half of their experimental plots with an extra 300 ppm of CO<sub>2</sub>. They found “the effect of elevated CO<sub>2</sub> on the number of flowering shoots (+24%) and seeds (+29%) at the community level was similar to above ground biomass response.” In terms of species functional groups, there was a 42% increase in the mean seed number of graminoids and a 33% increase in the mean seed number of forbs, but no change in legume seed numbers. In most species, mean seed weight also tended to be greater in plants grown in CO<sub>2</sub>-enriched air (+12%), and Thurig *et al.* note many studies have shown heavier seeds result in seedlings “more robust than seedlings from lighter seeds (Baskin and Baskin, 1998).”

Wang and Griffin (2003) grew dioecious white campion plants from seed to maturity in sand-filled pots maintained at optimum moisture and fertility conditions in environmentally controlled growth chambers in which the air was continuously maintained at CO<sub>2</sub> concentrations of either 365 or 730 ppm. In response to this doubling of the air’s CO<sub>2</sub> content, the vegetative mass of both male and female plants rose by approximately 39%. Reproductive mass rose by 82% in male plants and by 97% in females. In the female plants, this was accomplished, in part, by increases of 36% and 44% in the number and mass of seeds per plant, and by a 15% increase in the mass of individual seeds, in harmony with the findings of Jablonski *et al.* (2002), which they derived from a meta-analysis of the results of 159 CO<sub>2</sub> enrichment experiments conducted on 79 species of agricultural and wild plants. Dioecious plants comprise nearly half of all angiosperm families, so their allocation of a greater proportion of plant biomass to reproduction in a high-CO<sub>2</sub> world of the future bodes well for the biodiversity of Earth’s ecosystems.

Wang (2005) grew well-watered and -fertilized specimens of *Silene latifolia* from seed to maturity in pots within controlled environment chambers maintained at mean CO<sub>2</sub> concentrations of 386 and 696 ppm, documenting reproductive responses during growth and at final harvest. Then, the seeds produced by the plants in this experiment were used to grow a second generation of plants under the same environmental conditions in which the parent plants had been grown.

In the first experiment, the total reproductive biomass of the plants grown in CO<sub>2</sub>-enriched air was 32% greater than that of the plants grown in ambient air, as was the total number of fruit produced. In the second experiment, for seeds from female plants grown in ambient air, 55% of all emergence occurred within six days of sowing, and for seeds from plants grown in CO<sub>2</sub>-enriched air, 67% of total emergence occurred during the same period. In addition, 87% of the seeds from the elevated-CO<sub>2</sub>-grown plants ultimately germinated, whereas only 67% of the seeds from the ambient-CO<sub>2</sub>-grown plants did so. Finally, the plants produced a greater percentage of female progeny in the CO<sub>2</sub>-enriched air than in ambient-air (56.3% vs. 52.7%).

The combined effect of a greater number of seeds being produced per female plant, a higher percentage of seed germination, and more female-biased seed production in CO<sub>2</sub>-enriched air suggest white campion plants will fare well in a high-CO<sub>2</sub> world of the future, which might cause some to worry, as it is a rather cosmopolitan and somewhat weedy species. However, any plants of agricultural value with which it might compete also will be doing better in such conditions. Hence, the greater importance of this study is what it may imply about other dioecious species, especially in light of the fact that *Silene latifolia*, in the words of Wang, “has become a model system for studying sexual dimorphism and sex-determination mechanisms and is likely the most extensively studied dioecious species.”

Kuparinen *et al.* (2009) investigated the effects of a warming-induced increase in local convective turbulence (caused by a postulated 3°C increase in local temperature) on the long-distance dispersal (LDD) of seeds and pollen based on mechanistic models of wind dispersal (Kuparinen *et al.*, 2007) and population spread (Clark *et al.*, 2001) in a boreal forest of southern Finland. For light-seeded herbs, the group of researchers report spread rates increased by 35–42 m/yr (6.3–9.2%), and for heavy-seeded herbs the increase was 0.01–0.06 m/yr (1.9–6.7%). In addition, they note, “climate change driven advance-

ments of flowering and fruiting phenology can increase spread rates of plant populations because wind conditions in spring tend to produce higher spread rates than wind conditions later in the year.”

The four researchers from France, Germany, Israel, and the United States conclude—in addition to the obvious benefits of greater LDD (being better able to move toward more hospitable locations)—the increased wind dispersal of seeds and pollen may “promote geneflow between populations, thus increasing their genetic diversity and decreasing the risk of inbreeding depression,” citing Ellstrand (1992) and Aguilar *et al.* (2008). They further note, “increased gene flow between neighboring populations can accelerate adaptation to environmental change,” citing Davis and Shaw (2001) and Savolainen *et al.* (2007). The scientists report the “dispersal and spread of populations are widely viewed as a means by which species can buffer negative effects of climate change.”

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### 2.25.3 Trees

- Atmospheric CO<sub>2</sub> enrichment typically increases seed amount, size, weight, carbohydrate content, lipid content, and protein content, and also causes earlier production, earlier germination, and greater percent of germination.

LaDeau and Clark (2001) investigated how enriching the air with CO<sub>2</sub> impacts the reproductive capacity of trees by determining various responses of loblolly pines to atmospheric CO<sub>2</sub> enrichment at the Duke Forest in the Piedmont region of North Carolina (USA). In August 1996 three 30-m-diameter FACE rings had been set up around three groups of the 13-year-old trees to enrich the air about them to 200 ppm above the atmosphere’s normal background concentration; three identical FACE rings served as control plots. The trees were not mature at the start of the experiment, so they did not produce any cones until a few rare ones appeared in 1998. By the fall of 1999, however, the scientists found the CO<sub>2</sub>-enriched trees were twice as likely as trees growing in ambient air to be reproductively mature, and they produced three times more cones per tree. Similarly, the trees growing in the CO<sub>2</sub>-enriched air produced 2.4 times more cones in the fall of 2000, and from August 1999 through July 2000, the scientists collected three times

as many seeds in the CO<sub>2</sub>-fertilized FACE rings as in the control rings.

Hussain *et al.* (2001) also worked on this aspect of the Duke Forest FACE study. They report seeds collected from the CO<sub>2</sub>-enriched trees were 91% heavier than those collected from the trees growing in ambient air, and the CO<sub>2</sub>-enriched seeds had a lipid content 265% greater than that of the seeds produced on the ambient-treatment trees. They also noted the germination success for seeds developed under atmospheric CO<sub>2</sub> enrichment was more than three times greater than for control seeds developed at ambient CO<sub>2</sub>, regardless of germination CO<sub>2</sub> concentration. Seeds from the CO<sub>2</sub>-enriched trees germinated approximately five days earlier than their ambiently produced counterparts, again regardless of germination CO<sub>2</sub> concentration, and seedlings developing from seeds collected from CO<sub>2</sub>-enriched trees displayed significantly greater root lengths and needle numbers than seedlings developing from trees exposed to ambient air, also regardless of growth CO<sub>2</sub> concentration.

The propensity for elevated levels of atmospheric CO<sub>2</sub> to hasten the production of more plentiful seeds on the trees of this valuable timber species bodes well for naturally regenerating loblolly pine stands of the southeastern United States, where LaDeau and Clark report the trees “are profoundly seed-limited for at least 25 years.” Hence, as the air’s CO<sub>2</sub> content continues to climb, they conclude, “this period of seed limitation may be reduced.” In addition, the observations of Hussain *et al.* suggest loblolly pine trees in a CO<sub>2</sub>-enriched world of the future likely will display significant increases in their photosynthetic rates. Enhanced carbohydrate supplies resulting from this phenomenon likely will increase seed weight and lipid content. Such seeds consequently should exhibit significant increases in germination success, and their enhanced lipid supplies likely will lead to greater root lengths and needle numbers in developing seedlings. Consequently, when CO<sub>2</sub>-enriched loblolly pine seedlings become photosynthetically active, they likely will produce biomass at greater rates than those exhibited by seedlings growing under current CO<sub>2</sub> concentrations.

Five years later, LaDeau and Clark (2006a) conducted a follow-up study extending this work. They found “carbon dioxide enrichment affected mean cone production both through early maturation and increased fecundity,” so “trees in the elevated CO<sub>2</sub> plots produced twice as many cones between 1998 and 2004 as trees in the ambient plots.” In addition, the trees grown in elevated CO<sub>2</sub> “made the

transition to reproductive maturation at smaller [trunk] diameters,” and they “not only reached reproductive maturation at smaller diameters, but also at younger ages.” By 2004, for example, “roughly 50% of ambient trees and 75% of fumigated trees [had] produced cones.” In addition, “22% of the trees in high CO<sub>2</sub> produced between 40 and 100 cones during the study, compared with only 9% of ambient trees.”

“In this 8-year study,” the two researchers write, they found “previous short-term responses indeed persist,” in contradiction of those who downplay the immense biological benefits of atmospheric CO<sub>2</sub> enrichment. In addition, they note, “*P. taeda* trees that produce large seed crops early in their life span tend to continue to be prolific producers (Schultz, 1997),” and they conclude this fact, together with their findings, suggests “individual responses seen in this young forest may be sustained over their life span.”

LaDeau and Clark (2006b) additionally analyzed the seed and pollen responses of the loblolly pines to atmospheric CO<sub>2</sub> enrichment, finding the “trees grown in high-CO<sub>2</sub> plots first began producing pollen while younger and at smaller sizes relative to ambient-grown trees.” Cone pollen and airborne pollen grain abundances were significantly greater in the CO<sub>2</sub>-enriched stands. They write, “by spring 2005, 63% of all trees growing in high CO<sub>2</sub> had produced both pollen and seeds vs. only 36% of trees in the ambient plots.”

This propensity for elevated concentrations of atmospheric CO<sub>2</sub> to hasten and increase the production of pollen by this valuable timber species bodes well for naturally regenerated loblolly pine stands, which have a continuous range from Maryland south to Florida and west to Texas, where they currently are profoundly seed-limited for at least 25 years. In addition, the two researchers indicate precocious pollen production “could enhance the production of viable seeds by increasing the percentage of fertilized ovules,” and “more pollen disseminated from multiple-source trees may also increase rates of gene flow among stands, and could further reduce rates of self-pollination, indirectly enhancing the production of viable seeds.” Also of importance, in view of the negative twists some attempt to put on even overwhelmingly positive research findings, they state, “pine pollen is not a dangerous allergen for the public at large.”

Another major study of the reproductive responses of trees to elevated levels of atmospheric CO<sub>2</sub> was conducted at the Kennedy Space Center, Florida (USA), where in 1996 researchers enclosed

three species of scrub-oak (*Quercus myrtifolia*, *Q. chapmanii*, and *Q. geminata*) within 16 open-top chambers, half of which were maintained at 379 ppm CO<sub>2</sub> and half at 704 ppm. Five years later—in August, September, and October 2001—Stiling *et al.* (2004) counted the acorns on randomly selected twigs of each species, and in November they counted fallen acorns of each species within equal-size quadrates of ground area, additionally evaluating mean acorn weight, acorn germination rate, and degree of acorn infestation by weevils.

Acorn germination rate and degree of predation by weevils were unaffected by elevated CO<sub>2</sub>, and acorn size was enhanced by a small amount: 3.6% for *Q. myrtifolia*, 7.0% for *Q. chapmanii*, and 7.7% for *Q. geminata*. Acorn number responses, on the other hand, were enormous, but for only two of the three species, as *Q. geminata* did not register any CO<sub>2</sub>-induced increase in reproductive output, in harmony with its unresponsive overall growth rate. For *Q. myrtifolia*, however, Stiling *et al.* report, “there were four times as many acorns per 100 twigs in elevated CO<sub>2</sub> as in ambient CO<sub>2</sub> and for *Q. chapmanii* the increase was over threefold.” On the ground, the enhancement was greater still, with the researchers reporting “the number of *Q. myrtifolia* acorns per meter squared in elevated CO<sub>2</sub> was over seven times greater than in ambient CO<sub>2</sub> and for *Q. chapmanii*, the increase was nearly sixfold.”

Stiling *et al.* thus conclude “there will be large increases in seedling production in scrub-oak forests in an atmosphere of elevated CO<sub>2</sub>,” noting “this is important because many forest systems are ‘recruitment-limited’ (Ribbens *et al.*, 1994; Hubbell *et al.*, 1999).” This conclusion echoes that of LaDeau and Clark with respect to loblolly pines. Therefore, and if other trees behave similarly, the rising CO<sub>2</sub> content of Earth’s atmosphere likely will be a great boon to the regenerative prowess of the planet’s forests.

A third major study of CO<sub>2</sub> effects on seed production in trees was conducted at the FACE facility near Rhinelander, Wisconsin (USA), where young paper birch (*Betula papyrifera* Marsh.) seedlings were planted in 1997 and had been growing since 1998 in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 360 or 560 ppm, as well as at atmospheric ozone (O<sub>3</sub>) concentrations of either ambient or 1.5 times ambient. Darbah *et al.* (2007) collected many types of data pertaining to flowering, seed production, seed germination, and new seedling growth and development over the 2004–2006 growing seasons.

They found “elevated CO<sub>2</sub> had significant positive effect[s] on birch catkin size, weight, and germination success rate.” Specifically, “elevated CO<sub>2</sub> increased germination rate of birch by 110%, compared to ambient CO<sub>2</sub> concentrations, decreased seedling mortality by 73%, increased seed weight by 17% [and] increased [new seedling] root length by 59%.”

The six researchers found “the opposite was true of elevated O<sub>3</sub>”; it “decreased the germination rate of birch by 62%, decreased seed weight by 25%, and increased [new seedling] root length by [only] 15%.” In addition, they note, “the seeds produced under elevated O<sub>3</sub> had much less stored carbohydrate, lipids, and proteins for the newly developing seedling to depend on and, hence, the slow growth rate.” They also report “the total number of trees that flowered increased by 139% under elevated CO<sub>2</sub> [but only] 40% under elevated O<sub>3</sub>.” Likewise, they state, “with respect to the quantity of flowers produced, elevated CO<sub>2</sub> had [a] 262% increase, while that of elevated O<sub>3</sub> had [only a] 75% increase compared to the control treatment.”

Darbah *et al.* state their findings imply seedling recruitment in paper birch “will be enhanced under elevated CO<sub>2</sub> but reduced under elevated O<sub>3</sub>.”

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## 2.26 Starch

- As the air's CO<sub>2</sub> content rises, most of Earth's vegetation responds with enhanced rates of photosynthesis and greater production of carbohydrates. Many of these carbohydrates are exported from leaves and needles to provide energy or carbon skeletons to facilitate increased biomass production, after which remaining carbohydrates are generally converted into starch and stored within leaves or roots for future use.

CO<sub>2</sub>-induced starch accumulation is occurring in a variety of plants. Janssens *et al.* (1998) found a six-month period of atmospheric CO<sub>2</sub> exposure of 700 ppm caused a 90% increase in root starch accumulation in Scots pine seedlings relative to control seedlings exposed to ambient CO<sub>2</sub> of 350 ppm. Studying the same species, Kainulainen *et al.* (1998) report a significant enhancement in needle starch concentrations after three years of atmospheric CO<sub>2</sub> enrichment to 300 ppm above ambient. Similar results have been reported in tropical trees, where 10 (Lovelock *et al.*, 1998) and four (Wurth *et al.*, 1998) species exhibited approximate doublings of their leaf starch contents in response to a doubling of the atmospheric CO<sub>2</sub> content. In other tree studies, Rey and Jarvis (1998) noted a 100% CO<sub>2</sub>-induced increase in leaf starch contents of birch seedlings exposed to an atmospheric CO<sub>2</sub> concentration of 700 ppm, and Pan *et al.* (1998) report a 17-fold increase in this parameter for apple seedlings grown at an atmospheric CO<sub>2</sub> concentration of 1600 ppm.

Liu *et al.* (2005) found the combined effects of elevated CO<sub>2</sub> and ozone (O<sub>3</sub>) produced a significant increase in leaf nonstructural carbohydrates of three- and four-year-old European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst) seedlings under both mixed and monoculture conditions, which was similar to what was observed

under CO<sub>2</sub> enrichment alone. Hence, they conclude, “since the responses to the combined exposure were more similar to elevated pCO<sub>2</sub> than to elevated pO<sub>3</sub>, apparently elevated pCO<sub>2</sub> overruled the effects of elevated pO<sub>3</sub> on nonstructural carbohydrates.”

Kostiainen *et al.* (2006) also studied the combined effects of elevated carbon dioxide and ozone, examining fast-growing silver birch (*Betula pendula* Roth) clones grown out-of-doors at Suonenjoki, Finland, in open-top chambers maintained at ambient and 1.9x ambient CO<sub>2</sub> concentrations in combination with ambient and 1.5x ambient O<sub>3</sub> concentrations.

Among other findings, the five researchers report the elevated CO<sub>2</sub> treatment increased trunk starch concentration by 7%. Recognizing “the concentration of nonstructural carbohydrates (starch and soluble sugars) in tree tissues is considered a measure of carbon shortage or surplus for growth (Korner, 2003),” the Finnish researchers state the “starch accumulation observed under elevated CO<sub>2</sub> in this study indicates a surplus of carbohydrates produced by enhanced photosynthesis of the same trees (Riikonen *et al.*, 2004).” In addition, they report, “during winter, starch reserves in the stem are gradually transformed to soluble carbohydrates involved in freezing tolerance (Bertrand *et al.*, 1999; Piispanen and Saranpaa, 2001), so the increase in starch concentration may improve acclimation in winter.” The ongoing rise in the air's CO<sub>2</sub> content should be a boon to silver birch (and likely many other trees) in both summer and winter in both pristine and ozone-polluted air.

It should also be noted elevated CO<sub>2</sub> concentrations increase starch concentrations within non-woody herbaceous plants. Reid *et al.* (1998), for example, documented a doubling of the atmospheric CO<sub>2</sub> concentration led to a 148% increase in soybean leaf starch contents at both normal and elevated concentrations of ozone. Exposure to 1,000 ppm CO<sub>2</sub> caused a 10-fold increase in leaf starch concentrations of potato (Ludewig *et al.*, 1998).

It is therefore highly likely that rising atmospheric CO<sub>2</sub> concentrations will significantly boost starch production in plants, increasing the availability of an important raw material that can be metabolized to help sustain enhanced growth under a variety of stressful conditions.

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## 2.27 Stomatal Density

### 2.27.1 Herbaceous Plants

- In addition to sequentially reducing the openness of their stomata to restrict unnecessary water loss via excessive transpiration as the air's CO<sub>2</sub> content rises, some plants also reduce the density (number per area) of stomates on their leaves, but only to a certain degree, beyond which this latter phenomenon would be counterproductive.

As the air's CO<sub>2</sub> content rises, many plants reduce their stomatal apertures, because with more CO<sub>2</sub> in the air they don't need to open their stomates as wide as they do at lower atmospheric CO<sub>2</sub> concentrations to allow for sufficient inward diffusion of CO<sub>2</sub> for use in photosynthesis. As a result, plants growing in CO<sub>2</sub>-enriched air typically exhibit reduced rates of water loss via transpiration, smaller yield losses attributable to the indiscriminate uptake of air pollutants, and increased water-use efficiencies.

For much the same reason (and producing similar effects), many plants also reduce the density of stomates on the surfaces of their leaves at higher atmospheric CO<sub>2</sub> concentrations. This section reviews the findings of a few of the studies that have addressed this subject in non-woody herbaceous plants.

Case *et al.* (1998) collected 12 wild radish genotypes with a variety of stomatal indices and guard cell lengths in Maine (USA) and grew them to maturity in greenhouses maintained at atmospheric CO<sub>2</sub> concentrations of 370 and 680 ppm. They found the elevated CO<sub>2</sub> did not significantly affect stomatal index or guard cell length. Across all genotypes investigated, leaf surface characteristics essentially were unchanged by elevated CO<sub>2</sub>.

In the case of C<sub>4</sub> maize plants grown for 30 days in plexiglass chambers maintained at ambient or triple-ambient concentrations of atmospheric CO<sub>2</sub>, on the other hand, Maroco *et al.* (1999) determined the leaves of the CO<sub>2</sub>-enriched plants displayed approximately 10% fewer stomates per unit leaf area than the leaves of control plants growing in ambient air.

Why one of these plants exhibited no change in stomatal density as the air's CO<sub>2</sub> content was increased above the current ambient value, while the other exhibited a decrease, may perhaps be explained by the results reported by Gray *et al.* (2000). They identified a gene of the small mustard plant *Arabidopsis thaliana* that prevents decreases in the number density of leaf stomata in response to atmospheric CO<sub>2</sub> enrichment above a certain critical value of atmospheric CO<sub>2</sub> concentration. It can readily be appreciated why this is so, because decreases in stomatal density and conductance in response to rising atmospheric CO<sub>2</sub> concentrations (which are typically beneficial) cannot go on indefinitely, for there would ultimately come a point (likely different for different species) where further decreases in these plant properties become counterproductive, leading to a situation where the enhanced air-to-leaf CO<sub>2</sub> concentration gradient could not overcome the increased resistance of CO<sub>2</sub> entry into the leaf, causing the plant to die of carbon starvation, or where transpiration is reduced so much that leaf evaporative cooling cannot prevent plant death due to increased thermal stress.

Viewed in this light, the difference between the stomatal density responses of wild radish and maize likely derives from genetically programmed species-specific differences in the critical value of atmospheric CO<sub>2</sub> concentration at which the decline in stomatal density with increasing atmospheric CO<sub>2</sub> concentration is genetically terminated. Serna and Fenoll (2000) note, "plants seem to be well armed to cope with a further enrichment in atmospheric CO<sub>2</sub>," and genes such as the one discovered by Gray *et al.*—denoted HIC for high carbon dioxide—"should ensure that, at high CO<sub>2</sub> concentrations, changes in stomatal indices are kept to a minimum."

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### 2.27.2 Woody Plants

- As the air's CO<sub>2</sub> content rises, leaf stomatal densities (number per area) of woody plants range from no change at all to double-digit percentage decreases that cease after a few hundred ppm increase in CO<sub>2</sub>, beyond which this phenomenon becomes counterproductive.

As the air's CO<sub>2</sub> content rises, many plants reduce their stomatal apertures, because with more CO<sub>2</sub> in the air they don't need to open their stomates as wide as they do at lower atmospheric CO<sub>2</sub> concentrations to allow for sufficient inward diffusion of CO<sub>2</sub> for use in photosynthesis. As a result, plants growing in CO<sub>2</sub>-enriched air typically exhibit reduced rates of transpirational water loss, smaller productivity losses attributed to the uptake of aerial pollutants, and increased water use efficiency.

For much the same reason (and producing similar effects), many plants also reduce the density of stomates on their leaf surfaces at higher atmospheric CO<sub>2</sub> concentrations. This section reviews the findings of studies that addressed this subject in woody plants such as trees and shrubs.

Apple *et al.* (2000) grew two-year-old Douglas fir seedlings for three years in controlled-environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 350 or 550 ppm and ambient or elevated (ambient plus 4°C) air temperatures. They found neither elevated CO<sub>2</sub> nor elevated air temperature, acting alone or together, significantly affected needle stomatal density.

By contrast, in a study of the long-term effects of elevated CO<sub>2</sub> on various leaf properties of mature white oak trees growing at different distances from CO<sub>2</sub>-emitting springs in central Italy, Paoletti *et al.* (1998) found elevated CO<sub>2</sub> significantly decreased leaf stomatal density by a factor of nearly 1.5 as the air's CO<sub>2</sub> concentration rose from 350 to 750 ppm. From that point on, however, there were no further reductions in stomatal density, even for CO<sub>2</sub> concentrations as great as 2600 ppm.

Evaluating the impact of a 350-to-750 ppm increase in the atmosphere's CO<sub>2</sub> concentration, Lin *et al.* (2001) grew seven-year-old Scots pine seedlings in the field in open-top chambers for four years, applying no additional nutrients or irrigation waters to the soils in which the young trees were rooted. After

the fourth year of their experiment, a detailed analysis of their data revealed the extra CO<sub>2</sub> reduced needle stomatal density by an average of 7.4%, indicating Scots pines may be better able to conserve water and cope with periods of drought and water stress in a high-CO<sub>2</sub> world of the future.

Beerling *et al.* (1998) grew one-year old *Ginkgo biloba* saplings in greenhouses maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 560 ppm for three years. They found the leaves of plants grown at 560 ppm CO<sub>2</sub> exhibited significant reductions in both stomatal density (number of stomates per leaf area) and stomatal index (ratio of stomata to epidermal cells). Interestingly, the stomatal density of the CO<sub>2</sub>-enriched leaves was similar to that measured on fossilized *Ginkgo* leaves dating back to the Triassic and Jurassic time periods, and because the CO<sub>2</sub>-induced reductions in stomatal density and index did not impact rates of photosynthesis, it can be inferred the water-use efficiencies of ancient *Ginkgo* species were much higher than those of their modern counterparts. Consequently, as the CO<sub>2</sub> content of the air continues to rise, it may, as Beerling *et al.* remark, “contribute to restoring the function of this ‘living fossil’ species back to that more representative of its long geological history.”

Much like what has been learned about herbaceous plants, studies of the effects of atmospheric CO<sub>2</sub> enrichment on the leaf stomatal density of woody species reveal a wide range of responses, from no change at all to a double-digit percentage point decrease that ceases after a few hundred ppm increase in CO<sub>2</sub>. These changes have been found to be beneficial to the species that exhibit them, including the cessation of the response at a species-specific critical atmospheric CO<sub>2</sub> concentration.

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## 2.28 Sugars

- Atmospheric CO<sub>2</sub> enrichment typically increases plants’ ability to produce greater amounts of various forms of sugar.

Plants grown in CO<sub>2</sub>-enriched atmospheres nearly always exhibit increased photosynthetic rates, which typically lead to increased foliar concentrations of total nonstructural carbohydrates, including various sugars. Schortemeyer *et al.* (1999), for example, report significant increases in leaf total nonstructural carbohydrate contents in an Australian tree species exposed to twice-ambient atmospheric CO<sub>2</sub> concentrations for six weeks. Similarly, Wurth *et al.* (1998) found twice-ambient CO<sub>2</sub> levels increased leaf sugar concentrations by an average of 30% in four Panamanian tropical tree species. Just an eight-day exposure of apple seedlings to an atmospheric CO<sub>2</sub> concentration of 1,600 ppm led to a 38% increase in leaf sorbitol concentration.

In a study of *Pinus radiata* seedlings that had been growing for several years in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 360 and 650 ppm, Griffin *et al.* (2000) found needle sugar contents increased by 26%. Likewise, studying young spruce (*Picea abies*) seedlings for three years in growth chambers maintained at atmospheric CO<sub>2</sub> concentrations of 280, 420, and 560 ppm, Wiemken and Ineichen (2000) discovered that in late summer, fall, and winter, glucose concentrations in needles on the CO<sub>2</sub>-enriched trees were 40 to 50% higher than those of needles on trees subjected to ambient and sub-ambient CO<sub>2</sub> concentrations (420 and 280 ppm, respectively).

Atmospheric CO<sub>2</sub> enrichment also has been documented to increase sugar contents in plant organs other than leaves. Lake and Hughes (1999), for example, found a doubling of the air’s CO<sub>2</sub> concentration produced a 2.4-fold increase in nectar production in nasturtium flowers. And in the early flowering stage of *Cucumis melo* melons, Dag and Eisikowitch (2000) observed in a sector of a greenhouse maintained at a CO<sub>2</sub> concentration of 1,000 ppm throughout the morning, 400 ppm between 1300 and 1500 hours, and then 600 ppm until the next morning, nectar volumes per flower were

significantly higher than in the control sector of the greenhouse, sometimes by as much as 100%.

De Souza *et al.* (2008) grew sugarcane (*Saccharum officinarum* L.), one of Earth's most important sugar-producing plants, in pots within open-top chambers maintained at either ambient (~370 ppm) or elevated (~720 ppm) atmospheric CO<sub>2</sub> concentrations in the field under natural conditions at Sao Paulo, Brazil over a period of 50 weeks, during which time and at the end of the season, various plant physiological parameters and properties were measured. The plants grown in the elevated CO<sub>2</sub> chambers exhibited "an increase of about 30% in photosynthesis and 17% in height, and accumulated 40% more biomass in comparison with the plants grown at ambient CO<sub>2</sub>," and the CO<sub>2</sub>-enriched plants "also had lower stomatal conductance and transpiration rates (-37 and -32%, respectively), and higher water use efficiency (c.a. 62%)." In addition, the sucrose concentration in the sugarcane leaves rose from 2.18% in the ambient-treatment plants to 2.82% in the CO<sub>2</sub>-enriched plants, for a CO<sub>2</sub>-induced increase of 29%.

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## 2.29 Tannins

Atmospheric CO<sub>2</sub> enrichment significantly enhances the condensed tannin concentrations of the vast majority of Earth's trees and grasses, giving them stronger defenses against various herbivores both above and below ground. And by causing less methane to be released to the atmosphere via ruminants browsing on tree leaves and grass, this phenomenon should reduce the rate of rise of the air's methane concentration and thereby decrease methane-induced global warming.

Condensed tannins are naturally occurring secondary carbon compounds produced in the leaves of a number of different plants that often act to deter herbivorous insects. In New Zealand, the Legume Lotus is a good source of these substances, and scientists with the country's AgResearch Grasslands institute have determined sheep and cattle feeding on forage containing this plant may reduce their methane emissions by as much as 16%.

This latter finding is of special importance to New Zealanders, because the methane expelled by cattle and sheep, which is a by-product of the fermentation of feed in the rumen of these animals, accounts for close to 90% of the country's methane emissions. A significant reduction in such a large national source of one of the atmosphere's most potent greenhouse gases would go a long way toward reducing emissions of climate-altering substances, which would be, according to the press release that described this development, "very welcome." The release also stated consumption of tannins has "a variety of other animal-related benefits, such as improved milk yield, increased liveweight gain, decreased internal parasite burden and reduced occurrence of bloat, dags and fly strike."

It is also important to note ruminants, as they are called, comprise a great group of animals in addition to sheep and cattle. They are four-footed, hoofed,

even-toed, cud-chewing mammals that have a stomach consisting of four divisions or chambers; the group includes antelope, bison, buffalo, camel, deer, giraffe, goat, llama, etc. These animals eat a number of other types of plants that may also experience increases in leaf tannin production as the air's CO<sub>2</sub> content rises. The following sections investigate this subject as it applies to other types of plants.

### 2.29.1 Aspen Trees

- Because the amount of methane expelled by ruminants is an inverse function of the amount of condensed tannins contained in the foliage they consume, the increased tannin concentrations likely to exist in aspen foliage in a high-CO<sub>2</sub> world of the future should result in less methane being released to the atmosphere via ruminants browsing on the trees' leaves. This should help decrease methane-induced global warming.

King *et al.* (2001) grew aspen (*Populus tremuloides*) seedlings for five months in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 350 or 700 ppm; at the end of this period they collected and analyzed naturally senesced leaf litter. They found the elevated CO<sub>2</sub> of this particular study had no effect on the tannin concentration of leaf litter.

A substantially different result was obtained in an earlier study of aspen leaves conducted by McDonald *et al.* (1999), who grew aspen seedlings in controlled environment greenhouses maintained at either ambient (387 ppm) or elevated (696 ppm) CO<sub>2</sub> concentrations under conditions of either low or high light availability (half and full sunlight, respectively) for 31 days after the mean date of bud break. Under low light conditions the CO<sub>2</sub>-enriched seedlings exhibited an approximate 15% increase in leaf condensed tannin concentration, and under high light conditions the CO<sub>2</sub>-induced increase in leaf condensed tannin concentration was 175%.

In a much more complex study, Agrell *et al.* (2005) examined the effects of ambient and elevated concentrations of atmospheric CO<sub>2</sub> (360 and 560 ppm, respectively) and O<sub>3</sub> (35–60 ppb and 52–90 ppb, respectively) on the foliar chemistry of more mature aspen trees of two different genotypes (216 and 259) growing out-of-doors at the Aspen Free Air CO<sub>2</sub> Enrichment (FACE) facility near Rhinelander, Wisconsin (USA). They also studied the impacts of these effects on the host plant preferences of forest tent caterpillar larvae.

Agrell *et al.* report, “the only chemical component showing a somewhat consistent covariation with larval preferences was condensed tannins,” noting “the tree becoming relatively less preferred as a result of CO<sub>2</sub> or O<sub>3</sub> treatment was in general also the one for which average levels of condensed tannins were most positively (or least negatively) affected by that treatment.” The mean condensed tannin concentrations of the aspen 216 and 259 genotypes were 25% and 57% higher, respectively, under the elevated CO<sub>2</sub> and O<sub>3</sub> combination treatment compared to the ambient CO<sub>2</sub> and O<sub>3</sub> combination treatment.

In light of these findings, it is logical to presume that as atmospheric concentrations of CO<sub>2</sub> and O<sub>3</sub> continue to rise, the increase in condensed tannin concentration likely to occur in the foliage of aspen trees should lead to their leaves becoming less preferred for consumption by the forest tent caterpillar, which according to Agrell *et al.* is “an eruptive generalist defoliator in North American hardwood forests, causing extensive damage during outbreak years (Fitzgerald, 1995).” Also, because the amount of methane expelled by ruminants is an inverse function of the amount of condensed tannins in the foliage they consume, the increased aspen foliage tannin concentrations likely to exist in a high-CO<sub>2</sub> world of the future should result in less methane being released to the atmosphere via ruminants browsing on aspen foliage, thus decreasing an impetus for methane-induced global warming.

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### 2.29.2 Birch Trees

- Elevated concentrations of atmospheric CO<sub>2</sub> tend to increase leaf and fine-root tannin concentrations of birch trees, which tends to protect the trees' foliage from predation by voracious insect herbivores, protect the trees' roots from soil-borne pathogens and herbivores, enhance the sequestration of carbon in forest soils, and reduce methane emissions from ruminants that might consume the trees' foliage.

This section reviews how condensed tannin concentrations in the leaves and roots of paper birch (*Betula papyrifera* Marsh.) and silver birch (*Betula pendula* Roth) trees respond to atmospheric CO<sub>2</sub> enrichment with and without concomitant increases in atmospheric temperature and ozone concentrations.

Peltonen *et al.* (2005) studied the effect of doubled atmospheric CO<sub>2</sub> and O<sub>3</sub> concentrations on the accumulation of 27 phenolic compounds, including soluble condensed tannins, in the leaves of two European silver birch clones in seven-year-old soil-grown trees in central Finland exposed in open-top chambers for three growing seasons to ambient and twice-ambient atmospheric CO<sub>2</sub> and O<sub>3</sub> concentrations singly and in combination. They found the elevated CO<sub>2</sub> increased the concentration of soluble condensed tannins in the leaves of the trees by 19% and protected the leaves from elevated O<sub>3</sub> because, as they describe it, "all the O<sub>3</sub>-derived effects on the leaf phenolics and traits were prevented by elevated CO<sub>2</sub>."

Kuokkanen *et al.* (2003) grew two-year-old silver birch seedlings in both ambient air of 350 ppm CO<sub>2</sub> and air enriched to a CO<sub>2</sub> concentration of 700 ppm under conditions of either ambient temperature or ambient temperature plus 3°C for one full growing season in the field in closed-top chambers at the Mekrijärvi Research Station of the University of Joensuu in eastern Finland. Then, during the middle of the summer, when carbon-based secondary compounds of birch leaves are fairly stable, they picked several leaves from each tree and determined their condensed tannin concentrations, along with the concentrations of a number of other physiologically important substances. The concentration of total phenolics, condensed tannins, and their derivatives significantly increased in the leaves produced in the CO<sub>2</sub>-enriched air, as also was observed by Lavola and Julkunen-Titto (1994), Williams *et al.* (1994), Kinney *et al.* (1997), Bezemer and Jones (1998), and Kuokkanen *et al.* (2001). The extra 350 ppm of CO<sub>2</sub> nearly tripled condensed tannin concentrations in the

ambient-temperature air, while it increased their concentrations in the elevated-temperature air by a factor in excess of 3.5.

Parsons *et al.* (2003) grew two-year-old paper birch saplings in well-watered and fertilized 16-liter pots from early May until late August in glasshouse rooms maintained at either 400 or 700 ppm CO<sub>2</sub>. They found the concentration of condensed tannins in the fine roots of the saplings was increased by 27% in the CO<sub>2</sub>-enriched treatment. The researchers state, "the higher condensed tannin concentrations that were present in the birch fine roots may offer these tissues greater protection against soil-borne pathogens and herbivores."

Parsons *et al.* (2004) collected leaf litter samples from early September to mid-October beneath paper birch trees growing in ambient and CO<sub>2</sub>-enriched (to 200 ppm above ambient) FACE plots in northern Wisconsin (USA), which also were maintained under ambient and O<sub>3</sub>-enriched (to 19 ppb above ambient) conditions. Afterward, the leaf mass produced in each treatment was determined, sub-samples of the leaves were assessed for a number of chemical constituents (including nitrogen, which hastens leaf decay, and condensed tannins, which retard decay). The remaining leaves were placed in 1-mm-aperture litterbags made of fiberglass cloth and left to decay on the ground for the next 12 months under the same atmospheric conditions in which they were produced. At the conclusion of the one-year litter-exposure period, the researchers measured the mass of remaining litter and determined the time required to achieve 95% mass loss.

Under ambient O<sub>3</sub> conditions, the nitrogen concentrations of the leaves in the CO<sub>2</sub>-enriched plots at the time of litterfall were 31% less than those of the leaves in the ambient-CO<sub>2</sub> plots, and condensed tannin concentrations were 64% greater in the CO<sub>2</sub>-enriched plots. Similarly, under the O<sub>3</sub>-enriched conditions, leaf nitrogen concentrations were 32% less, and concentrations of condensed tannins were 99% greater.

These observations suggest leaf decay rates in the CO<sub>2</sub>-enriched plots should be lower than in the ambient-CO<sub>2</sub> plots, and the mass-loss rates determined at the end of the one-year exposure period bore out this expectation. Parsons *et al.* report, "for control litter, 5% of mass remained after 3.6 years, while CO<sub>2</sub>-enriched litter took ~4.5 years to turn over 95% of its mass." Hence, it could take 25% more time (4.5 years / 3.6 years) to lose an equivalent percentage of paper birch leaf litter from CO<sub>2</sub>-enriched forests, independent of the air's O<sub>3</sub> concentration. In addition,

the CO<sub>2</sub>-enriched trees “attained greater size, and a greater degree of canopy closure, and contributed more litterfall to the development of [the] forest floor than did trees in the control rings,” making it clear the ongoing rise in the atmosphere’s CO<sub>2</sub> concentration should greatly augment the sequestration of carbon by paper birch tree stands as the air’s CO<sub>2</sub> content climbs.

Also working at the Wisconsin FACE site, Agrell *et al.* (2005) examined the effects of ambient and elevated concentrations of atmospheric CO<sub>2</sub> (360 and 560 ppm) and O<sub>3</sub> (35–60 ppb and 52–90 ppb) on the foliar chemistry of paper birch trees, as well as the impacts of these effects on the host plant preferences of forest tent caterpillar larvae. They found the mean condensed tannin concentration of the birch tree leaves was 18% greater in the elevated CO<sub>2</sub> and O<sub>3</sub> treatment than in the ambient CO<sub>2</sub> and O<sub>3</sub> treatment. In addition, “the only chemical component showing a somewhat consistent covariation with larval preferences was condensed tannins,” and “the tree becoming relatively less preferred as a result of CO<sub>2</sub> or O<sub>3</sub> treatment was in general also the one for which average levels of condensed tannins were most positively (or least negatively) affected by that treatment.”

In light of these findings, it is logical to presume the rising atmospheric concentrations of CO<sub>2</sub> and O<sub>3</sub> and consequent increase in condensed tannin concentration likely to occur in the foliage of birch trees should lead to their leaves becoming less preferred for consumption by the forest tent caterpillar, which according to Agrell *et al.* is “an eruptive generalist defoliator in North American hardwood forests, causing extensive damage during outbreak years (Fitzgerald, 1995).” Also, because the amount of methane expelled by ruminants is an inverse function of the condensed tannin concentration of the foliage they consume, the higher birch-foliage tannin concentrations likely to prevail in a high-CO<sub>2</sub> world of the future should result in less methane being released to the atmosphere via ruminants browsing on the foliage of birch trees, reducing that impetus for methane-induced global warming.

Elevated concentrations of atmospheric CO<sub>2</sub> tend to increase leaf and fine-root tannin concentrations of birch trees, which in turn tends to protect the trees’ foliage from predation by voracious insect herbivores, protect the trees’ roots from soil-borne pathogens and herbivores, enhance the sequestration of carbon in forest soils, and reduce methane emissions from ruminants that consume the trees’ foliage.

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### 2.29.3 Oak Trees

- The large increase of condensed tannin concentrations in oak tree foliage produced in CO<sub>2</sub>-enriched air results in methane emissions

from ruminants feeding on such foliage being lower than methane emissions from ruminants feeding on foliage grown in non-CO<sub>2</sub>-enriched air, and foliage better able to resist herbivore attacks.

This section examines how rising air temperatures and atmospheric CO<sub>2</sub> concentrations affect the leaf tannin concentrations of several oak tree species, including myrtle oak (*Quercus myrtifolia* Wild.), pedunculate oak (*Quercus robur* L.), sand live oak (*Quercus geminata* Small), and Chapman's oak (*Quercus chapmanii* Sargent).

Dury *et al.* (1998) grew four-year-old pedunculate oak trees in pots within greenhouses maintained at ambient and twice-ambient atmospheric CO<sub>2</sub> concentrations in combination with ambient and elevated (ambient plus 3°C) air temperatures for approximately one year. The elevated CO<sub>2</sub> concentration had only minor and contrasting direct effects on leaf palatability, including a temporary increase in foliar phenolic concentrations and decreases in leaf toughness and nitrogen content. The elevated temperature treatment, on the other hand, significantly reduced leaf palatability, because oak leaf toughness increased as a consequence of temperature-induced increases in condensed tannin concentrations. Hence the five researchers conclude, “a 3°C rise in temperature might be expected to result in prolonged larval development, increased food consumption, and reduced growth” for herbivores feeding on oak leaves in a CO<sub>2</sub>-enriched and warmer world of the future.

Cornelissen *et al.* (2003) studied fluctuating asymmetry in the leaves of two species of sclerophyllous oaks—myrtle oak (*Quercus myrtifolia*) and sand live oak (*Quercus geminata*)—that dominate a native scrub-oak community at the Kennedy Space Center, Titusville, Florida (USA), which has served as the base of operations for a number of important open-top-chamber investigations of the effects of a 350-ppm increase in the air's CO<sub>2</sub> concentration on this unique ecosystem. Fluctuating asymmetry is the terminology used to describe small variations from perfect symmetry in otherwise bilaterally symmetrical characters in an organism (Moller and Swaddle, 1997); such asymmetry is believed to arise as a consequence of developmental instabilities experienced during ontogeny that may result from various stresses, including both genetic and environmental factors (Moller and Shykoff, 1999).

Based on measurements of distances from the leaf midrib to the left and right edges of the leaf at its

widest point and leaf areas on the left and right sides of the leaf midrib, Cornelissen *et al.* determined “asymmetric leaves were less frequent in elevated CO<sub>2</sub>, and, when encountered, they were less asymmetric than leaves growing under ambient CO<sub>2</sub>.” In addition, they found “*Q. myrtifolia* leaves under elevated CO<sub>2</sub> were 15.0% larger than in ambient CO<sub>2</sub> and *Q. geminata* leaves were 38.0% larger in elevated CO<sub>2</sub> conditions.” They also determined “elevated CO<sub>2</sub> significantly increased tannin concentration for both *Q. myrtifolia* and *Q. geminata* leaves” and “asymmetric leaves contained significantly lower concentrations of tannins than symmetric leaves for both *Q. geminata* and *Q. myrtifolia*.”

Commenting on their two primary findings—reduced percentages of leaves experiencing asymmetry in the presence of elevated levels of atmospheric CO<sub>2</sub> and the lesser degree of asymmetry exhibited by affected leaves in the elevated CO<sub>2</sub> treatment—Cornelissen *et al.* write, “a possible explanation for this pattern is the fact that, in contrast to other environmental stresses, which can cause negative effects on plant growth, the predominant effect of elevated CO<sub>2</sub> on plants is to promote growth with consequent reallocation of resources (Docherty *et al.*, 1996).” Another possibility they discuss “is the fact that CO<sub>2</sub> acts as a plant fertilizer,” and, as a result, “elevated CO<sub>2</sub> ameliorates plant stress compared with ambient levels of CO<sub>2</sub>,” which is one of the well-documented biological benefits of atmospheric CO<sub>2</sub> enrichment (Idso and Idso, 1994).

With respect to the ancillary finding of CO<sub>2</sub>-induced increases in tannin concentrations in the leaves of both oak species (a mean increase of approximately 35% for *Q. myrtifolia* and 43% for *Q. geminata*), this phenomenon may provide the two species with greater protection against herbivores, and part of that protection may be associated with the observed CO<sub>2</sub>-induced reductions in the amount and degree of asymmetry in the leaves of the CO<sub>2</sub>-enriched trees. Consistent with this hypothesis, for example, Stiling *et al.* (1999, 2003) found higher abundances of leaf miners in the leaves of the trees in the ambient CO<sub>2</sub> chambers, where asymmetric leaves were more abundant, and in the current study it was determined leaf miners attacked asymmetric leaves more frequently than would be expected by chance alone in both CO<sub>2</sub> treatments.

In a subsequent study conducted at the Kennedy Space Center's scrub-oak community, Hall *et al.* (2005b) evaluated foliar quality and herbivore damage in three oaks (*Q. myrtifolia*, *Q. chapmanii*, and *Q. geminata*) plus the nitrogen-fixing legume

*Galactia elliottii* at three-month intervals from May 2001 to May 2003, at which times samples of undamaged leaves were removed from each of the four species in all chambers and analyzed for various chemical constituents, and 200 randomly selected leaves of each species in each chamber were scored for the presence of six types of herbivore damage. The data indicated for condensed tannins, hydrolyzable tannins, total phenolics and lignin, in all four species there were always greater concentrations of all four leaf constituents in the CO<sub>2</sub>-enriched leaves, with across-species mean increases of 6.8% for condensed tannins, 6.1% for hydrolyzable tannins, 5.1% for total phenolics, and 4.3% for lignin. There also were large CO<sub>2</sub>-induced decreases in all leaf damage categories among all species: chewing (-48%), mines (-37%), eye spot gall (-45%), leaf tier (-52%), leaf mite (-23%), and leaf gall (-16%). Hall *et al.* conclude the changes in leaf chemical constituents and herbivore damage “suggest that damage to plants may decline as atmospheric CO<sub>2</sub> levels continue to rise.”

Largely overlapping the investigation of Hall *et al.* (2005b), Hall *et al.* (2005a) evaluated the effects of the Kennedy Space Center experiment’s extra 350 ppm of CO<sub>2</sub> on litter quality and herbivore activity, and their interactions, over the three-year-period 2000–2002. They found “changes in litter chemistry from year to year were far larger than effects of CO<sub>2</sub> or insect damage, suggesting that these may have only minor effects on litter decomposition.” The one exception to this finding was “condensed tannin concentrations increased under elevated CO<sub>2</sub> regardless of species, herbivore damage, or growing season,” rising by 11% in 2000, 18% in 2001, and 41% in 2002 as a result of atmospheric CO<sub>2</sub> enrichment, as best as can be determined from the researchers’ bar graphs. The five scientists also report “lepidopteran larvae can exhibit slower growth rates when feeding on elevated CO<sub>2</sub> plants (Fajer *et al.*, 1991) and become more susceptible to pathogens, parasitoids, and predators (Lindroth, 1996; Stiling *et al.*, 1999),” noting further that at their field site, “which hosts the longest continuous study of the effects of elevated CO<sub>2</sub> on insects, herbivore populations decline markedly under elevated CO<sub>2</sub> (Stiling *et al.*, 1999, 2002, 2003; Hall *et al.*, 2005b).”

The evidence thus suggests the large and continuous enhancement of condensed tannin concentrations in oak tree foliage produced in CO<sub>2</sub>-enriched air will help reduce greenhouse gas-induced global warming because methane emissions from ruminants feeding on foliage rich in condensed

tannins tend to be lower than when those ruminants feed on foliage of lower tannin concentration. In addition, the marked tannin-induced declines in herbivore populations observed in CO<sub>2</sub>-enriched open-top-chamber studies indicate the ongoing increase in atmospheric CO<sub>2</sub> will improve plants’ resistance to herbivore attacks, a highly beneficial outcome.

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#### 2.29.4 Other

- Atmospheric CO<sub>2</sub> enrichment significantly enhances the concentrations of condensed tannins in the vast majority of the planet's trees and grasses, providing them with stronger defenses against various herbivores both above and below ground. Increasing atmospheric CO<sub>2</sub> levels also may be helping to reduce the rate-of-rise of the air's methane concentration.

Ruminants—sheep, cattle, antelope, bison, buffalo, camel, deer, giraffe, goat, llama, and more—eat a wide variety of plants, many of which undergo increases in leaf tannin production as the air's CO<sub>2</sub> content rises, as has been found to be true for a number of different species, including both deciduous and evergreen trees, as described by Lindroth *et al.* (1993, 1995), Traw *et al.* (1996), and Hattenschwiler and Schafellner (1999), and a number of species of grass, as described by (Goverde *et al.*, 2002).

With respect to how widespread CO<sub>2</sub>-induced increases in foliar phenolics and tannins are, and the magnitudes of their increases, almost all relevant knowledge initially came from studies of temperate-region trees, the leaf phenolic concentrations of which had been shown to rise by 20–60% in response to a doubling of the air's CO<sub>2</sub> content (Koricheva *et al.*, 1998; Peñuelas and Estiarte, 1998; McDonald *et al.*, 1999; Agrell *et al.*, 2000; Hartley *et al.*, 2000). This knowledge base was vastly enhanced by the experiment of Coley *et al.* (2002), which focused on nine species of tropical trees. The trees were rooted in the ground and grown in their natural environment (near the Smithsonian Tropical Research Institute's experiment site in central Panama), rather than being planted in pots and grown in greenhouses. This point

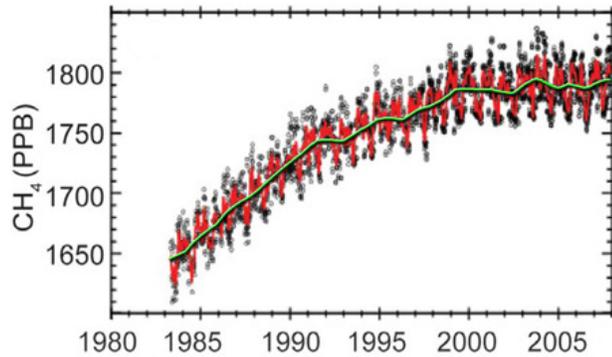
is especially important because nonnatural environments can lead to results different from those obtained with open-top chambers or FACE facilities constructed around trees growing out-of-doors in the absence of artificial root restrictions (O'Neil and Norby, 1996).

Eight of the nine species studied in Coley *et al.*'s six-month open-top chamber experiment exhibited positive leaf phenolic/tannin responses to a doubling of the atmosphere's CO<sub>2</sub> content, the largest of which was a concentration increase of 119%. The single negative response was a 27% decline, and the mean response of all nine species was an increase of 48%. These results are comparable with those obtained for temperate-region trees, and they provide the basis for Coley *et al.*'s primary conclusion; i.e., that although “both temperate and tropical trees show large interspecific variation in the extent of their response to CO<sub>2</sub> ... the overwhelming pattern is for an increase in phenolics by approximately 50%.”

For four years, Kelly *et al.* (2010) grew six-year-old quaking aspen (*Populus tremuloides*) clones, two-year-old white willow (*Salix alba*) clones, and two-year-old sugar maple (*Acer saccharum*) siblings out-of-doors at the University of Michigan Biological Station in northern Michigan (USA) in open-bottom root boxes enclosed within clear-plastic-wall open-top chambers continuously supplied throughout the growing season (from May until leaf senescence in November) with either ambient-CO<sub>2</sub>-air (360 ppm) or elevated-CO<sub>2</sub>-air (720 ppm). At the conclusion of the four-year period, fallen leaves were collected, dried, and analyzed for simple phenolic and condensed tannin concentrations. Kelly *et al.*'s tabular results indicate the 360-ppm CO<sub>2</sub> increase boosted the simple phenolics concentrations of the aspen, maple, and willow leaves by 16, 30, and 22%, respectively, and it boosted their condensed tannin concentrations by 60, 85, and 26%, respectively. And because both foliar phenolics and condensed tannins often enhance plant resistance to herbivore and pathogen attack, plus the fact that ruminants browsing on foliage containing condensed tannins seem to have a tendency to expel less methane to the atmosphere, the increased concentrations of these substances in the leaves of the trees grown in CO<sub>2</sub>-enriched air bodes well for the health of the trees themselves and for some amelioration of CO<sub>2</sub>- and methane-induced global warming.

These findings suggest many wild and domesticated animals may be participating in this important natural “program” for reducing methane emissions to the atmosphere. They may also be at

least partially responsible for the reduced rate of rise of the atmosphere's methane concentration (as shown in Figure 2.29.4.1) over the past few decades. If so, one could expect to see more of the same as the air's CO<sub>2</sub> content continues to rise; for the biosphere appears to take care of its own, as demonstrated by this unique negative feedback phenomenon.



**Figure 2.29.4.1.** Trace gas mole fractions of methane (CH<sub>4</sub>) as measured at Mauna Loa, Hawaii. Adapted from Schnell and Dlugokencky (2008).

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## 2.30 Thylakoid Membranes

- Thylakoid membranes of chloroplasts perform a host of important functions that influence the process of carbon fixation in plants. In CO<sub>2</sub>-

enriched air, they generally perform these functions more efficiently, enabling plants to overcome some of the deleterious effects of a host of plant stresses, including insufficient soil moisture, abnormally cold temperatures, abnormally hot temperatures, and ozone pollution.

Nearly all plants respond positively to increases in the air's CO<sub>2</sub> content by increasing their rates of photosynthesis, partly as a consequence of decreases in photorespiration and increases in the operating efficiency of the primary carboxylating enzyme rubisco. Less research has addressed the direct effects of elevated CO<sub>2</sub> on thylakoid membranes, the membranes within chloroplasts that contain chlorophyll and other pigments that absorb sunlight, together with the specialized protein complexes of photosystem II that play an integral role in the light-dependent reactions of photosynthesis that convert sunlight into usable cellular energy. This section reviews the findings of papers investigating how elevated CO<sub>2</sub> may impact thylakoid membranes and thereby influence the overall process of carbon fixation in plants.

After growing alfalfa in open-top chambers at ambient (340 ppm) and enriched (600 ppm) atmospheric CO<sub>2</sub> concentrations with ample soil moisture for 25 days, followed by inadequate levels of soil moisture for five additional days, Sgherri *et al.* (1998) determined the plants grown in the elevated CO<sub>2</sub> treatment maintained greater leaf chlorophyll contents and lipid-to-protein ratios in their thylakoid membranes, especially under conditions of water stress. When water was withheld, for example, leaf chlorophyll content dropped by a mere 6% at 600 ppm CO<sub>2</sub>, whereas it plummeted by approximately 30% at 340 ppm. Leaf lipid contents in the plants grown in the CO<sub>2</sub>-enriched air were about 22 and 83% higher than those measured in the plants grown in the ambient air treatment during the periods of ample and insufficient soil moisture, respectively. In addition, in the high CO<sub>2</sub> treatment the average degree of unsaturation of the two most important thylakoid membrane lipids was approximately 20 and 37% greater than it was in plants grown at 340 ppm CO<sub>2</sub> during times of adequate and inadequate soil moisture, respectively.

With respect to the implications of these observations, it is generally believed that the greater concentrations of thylakoid lipids typically observed at elevated atmospheric CO<sub>2</sub> concentrations, plus their enhanced degree of unsaturation, may allow thylakoid

membranes to maintain a more fluid and stable environment, which is critical during periods of water stress in enabling plants to continue photosynthetic carbon uptake. These effects are so important, some researchers suggest adaptive plant responses such as these may allow plants to better cope with *any* altered environmental condition that produces stress.

The study of Tuba *et al.* (1998) provides a good example of this phenomenon as it pertains to extreme water stress. Detached leaves of *Xerophyta scabrida* (a woody shrub that grows in arid regions of east Africa) were rehydrated and regreened in air of 350 and 700 ppm CO<sub>2</sub>, after which they were allowed to desiccate. The elevated CO<sub>2</sub> did not affect the amount of chlorophyll, the functioning of the thylakoid membranes, or the time to complete drying during desiccation. However, it allowed positive photosynthetic carbon gains in the shrub's leaves to continue three times longer than in leaves exposed to ambient air, which resulted in the CO<sub>2</sub>-enriched leaves gaining more than 10 times the amount of carbon over the period of desiccation than was gained by the leaves exposed to normal air.

Tuba *et al.* also studied the effects of desiccation on the carbon balance of a moss and a lichen under the same two atmospheric CO<sub>2</sub> concentrations, observing similar response patterns in both plants. Positive photosynthetic carbon gains were maintained 14% longer with atmospheric CO<sub>2</sub> enrichment, and total assimilation during the dry-down in elevated CO<sub>2</sub> was increased by 52 and 69% in the lichen and moss, respectively.

Investigating responses to chilling, Hugly and Somerville (1992) worked with wild-type *Arabidopsis thaliana* and two mutants deficient in thylakoid lipid unsaturation. They found "chloroplast membrane lipid polyunsaturation contributes to the low-temperature fitness of the organism" and "is required for some aspect of chloroplast biogenesis." When lipid polyunsaturation was low, for example, they observed "dramatic reductions in chloroplast size, membrane content, and organization in developing leaves." Furthermore, they found a positive correlation "between the severity of chlorosis in the two mutants at low temperatures and the degree of reduction in polyunsaturated chloroplast lipid composition."

Kodama *et al.* (1994) demonstrated the low-temperature-induced suppression of tobacco leaf growth and concomitant induction of chlorosis observed in wild-type plants was much less evident in transgenic plants containing a gene that allowed for greater expression of unsaturation in the fatty acids of leaf lipids. They conclude substantially unsaturated

fatty acids “are undoubtedly an important factor contributing to cold tolerance.”

Moon *et al.* (1995) found heightened unsaturation of the membrane lipids of chloroplasts stabilized the photosynthetic machinery of transgenic tobacco plants against low-temperature photoinhibition “by accelerating the recovery of the photosystem II protein complex.” Also working with transgenic tobacco plants, Kodama *et al.* (1995) showed increased fatty acid desaturation is one of the prerequisites for normal leaf development at low, nonfreezing temperatures, and Ishizaki-Nishizawa *et al.* (1996) demonstrated transgenic tobacco plants with a reduced level of saturated fatty acids in most membrane lipids “exhibited a significant increase in chilling resistance.”

These observations are laden with significance for Earth’s agro-ecosystems because many economically important crops, such as rice, maize, and soybeans, are classified as chilling-sensitive; they experience injury or death at temperatures between 0 and 15°C (Lyons, 1973). But if atmospheric CO<sub>2</sub> enrichment enhances their production and degree-of-unsaturation of thylakoid lipids, as it does in alfalfa, a continuation of the ongoing rise in the air’s CO<sub>2</sub> content could increase the abilities of these critically important agricultural species to withstand periodic exposure to debilitating low temperatures. This phenomenon could provide the extra boost in food production that will be needed to sustain the planet’s increasing human population in the future (Wallace, 2000; Tilman *et al.*, 2001).

Earth’s natural ecosystems also would benefit from a CO<sub>2</sub>-induced increase in thylakoid lipids containing more-highly-unsaturated fatty acids. Many plants of tropical origin, for example, suffer cold damage when temperatures fall below 20°C (Graham and Patterson, 1982), and with the improved lipid characteristics provided by the ongoing rise in the air’s CO<sub>2</sub> content, such plants would be able to expand their ranges both poleward and upward in a higher-CO<sub>2</sub> world, significantly increasing ecosystem biodiversity along the way.

At the other end of the biologically tolerable temperature spectrum is heat stress. Taub *et al.* (2000) note electron transport through photosystem II is the most heat-sensitive component of the photosynthetic process, and any reductions in electron transport through this thylakoid-membrane-bound protein complex invariably lead to reductions in photosynthetic carbon uptake and reduced growth potential. Hence, they conducted several experiments on herbaceous, woody, monocot, and dicot species (to

assess the degree of universality of any response that might be detected) in controlled environment chambers, greenhouses, and FACE plots to examine the photosynthetic responses of this wide array of plants to acute heat stress under ambient and elevated CO<sub>2</sub> concentrations ranging from 550 to 1,000 ppm.

Of the 16 plant species studied, all but one displayed greater photochemical efficiencies of photosystem II when growing in CO<sub>2</sub>-enriched air as opposed to ambient air when exposed to high air temperatures. The air temperatures that caused a 50% reduction in the maximum efficiency of photosystem II were nearly one degree Celsius higher for plants grown in elevated CO<sub>2</sub> air than for plants grown in ambient air. In other words, elevated CO<sub>2</sub> almost universally allowed more electrons to flow through photosystem II, thereby laying the foundation for greater photosynthetic rates. In an extended experiment, rates of net photosynthesis measured at 40°C in CO<sub>2</sub>-enriched cucumbers were 3.2 times greater than those exhibited by plants grown in ambient air and exposed to the same air temperature.

Another stress to which many plants are routinely exposed is elevated atmospheric ozone concentrations. Oksanen *et al.* (2001) grew aspen clones with varying degrees of ozone tolerance together with sugar maple and paper birch trees for three years in 30-m diameter FACE plots maintained at atmospheric CO<sub>2</sub> concentrations of 360 and 560 ppm with and without exposure to elevated ozone concentrations (1.5 times ambient) to study the interactive effects of these two trace gases on leaf ultrastructure.

In the birch trees, the negative effects of ozone on leaf ultrastructure were minor, and injuries to thylakoid membranes were partially ameliorated by exposure to elevated CO<sub>2</sub>. In the aspen clones, ozone exposure caused more significant structural injuries to thylakoid membranes and the stromal compartment within chloroplasts, but these injuries also were largely ameliorated by atmospheric CO<sub>2</sub> enrichment. Leaf thickness, mesophyll tissue thickness, the amount of chloroplasts per unit cell area, and the amount of starch in chloroplasts all were lower in the high ozone treatment, but simultaneous exposure of the ozone-stressed trees to elevated CO<sub>2</sub> more than compensated for the ozone-induced problems. As tropospheric ozone concentrations continue to rise, therefore, they likely will pose a problem for regenerating aspen and birch trees by negatively affecting chloroplast ultrastructure at the site of carbon fixation, which likely will decrease their productivity and growth. However, if the atmospheric CO<sub>2</sub> concentration also continues to rise, these

negative effects will be either partly, completely, or more than completely ameliorated, thus stimulating productivity and growth to varying degrees within these species.

The studies reviewed above suggest atmospheric CO<sub>2</sub> enrichment may constitute a powerful remedy for all sorts of environmental ailments that afflict plants and have their origin in stress-induced problems associated with the functioning of the thylakoid membranes of the plants' chloroplasts.

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## 2.31 Transpiration

### 2.31.1 Herbaceous Plants

If the atmosphere's CO<sub>2</sub> content continues to rise as it has over the past few decades, the world's farmers should be able to produce substantially more food on each hectare of land with little or no change in per-hectare water requirements. In the case of C<sub>4</sub> crops, higher yields may be produced with smaller amounts of water, even in the face of higher temperatures. And most herbaceous non-crop plants should respond to increases in the air's CO<sub>2</sub> concentration in like manner, leading to greater soil moisture content in CO<sub>2</sub>-enriched ecosystems and a positive effect on plant growth.

#### 2.31.1.1 Crops

- If the atmosphere's CO<sub>2</sub> content continues to rise as it has over the past few decades, the world's farmers should be able to produce substantially more food on each hectare of land with little or no change in per-hectare water requirements. Higher yields of C<sub>4</sub> crops may be produced with smaller amounts of water, even in higher temperatures.

Most plants respond to increases in the atmosphere's CO<sub>2</sub> content by reducing their leaf stomatal conductances, which typically leads to reduced rates of transpirational water loss. The resultant water savings, in turn, often lead to greater soil moisture content in CO<sub>2</sub>-enriched ecosystems, with positive effects on plant water status and growth. This section

reviews the results of studies of both C<sub>3</sub> and C<sub>4</sub> crops that treat various aspects of this CO<sub>2</sub>-induced multistage interaction.

Dong-Xiu *et al.* (2002) grew spring wheat in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm and three levels of soil moisture (40, 60, and 80% of field capacity). In addition to increasing rates of net photosynthesis by 48, 120, and 97% at low, medium, and high soil water contents, this doubling of the air's CO<sub>2</sub> concentration reduced rates of transpiration by 56, 53, and 63%, respectively, in the three soil water treatments.

De Costa *et al.* (2003) grew two crops of rice in the field in Sri Lanka—from January to March (the *maha* season) and from May to August (the *yala* season)—in open-top chambers maintained at either ambient or ambient plus 200 ppm CO<sub>2</sub>. Leaf net photosynthetic rates were significantly higher in the CO<sub>2</sub>-enriched chambers than in the ambient-air chambers: 51–75% greater in the *maha* season and 22–33% greater in the *yala* season. In addition, leaf stomatal conductances exhibited CO<sub>2</sub>-induced reductions of 15–52% in the *maha* season and 13–19% in the *yala* season. However, because of the significantly greater leaf area in the CO<sub>2</sub>-enriched chambers, total canopy transpiration rate per unit land area did not differ significantly between the two CO<sub>2</sub> treatments. Nevertheless, leaf water potentials were higher (less negative, and therefore more beneficial) in the CO<sub>2</sub>-enriched chambers.

Vu (2005) grew peanuts from seed to maturity in greenhouses maintained at atmospheric CO<sub>2</sub> concentrations of 360 and 720 ppm and at air temperatures 1.5 and 6.0°C above outdoor air temperatures. Although rubisco protein content and activity were down-regulated by elevated CO<sub>2</sub>, the rubisco photosynthetic efficiency of the elevated-CO<sub>2</sub> plants “was 1.3- to 1.9-fold greater than that of the ambient-CO<sub>2</sub> plants at both growth temperatures.” He also found “leaf soluble sugars and starch of plants grown at elevated CO<sub>2</sub> were 1.3- and 2-fold higher, respectively, than those of plants grown at ambient CO<sub>2</sub>.” Finally, the leaf transpirational water loss of the elevated-CO<sub>2</sub> plants compared to that of the ambient-CO<sub>2</sub> plants was 12% less at near-ambient temperatures and 17% less in the higher temperature regime.

Malmstrom and Field (1997) grew individual oat plants for two months in pots placed within phytocells having atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm. One-third of the plants were infected with the barley yellow dwarf virus that plagues more than 150 plant species, including all major cereal crops.

The elevated CO<sub>2</sub> stimulated rates of net photosynthesis in all plants, with the greatest percentage increase occurring in diseased individuals (48% vs. 34%), and it decreased stomatal conductance by 50% in infected plants and 34% in healthy ones, thus reducing transpirational water losses. Together, these phenomena contributed to a CO<sub>2</sub>-induced doubling of the instantaneous water use efficiency in healthy plants and a 2.7-fold increase in diseased plants.

Leakey *et al.* (2004) grew corn in their SoyFACE facility in the heart of the U.S. Corn Belt while exposing different parts of the field to atmospheric CO<sub>2</sub> concentrations of 354 and 549 ppm during a year of summer rainfall “very close to the 50-year average for this site, indicating that the year was not atypical or a drought year.” On five days during the growing season (11 and 22 July, 9 and 21 August, and 5 September), they measured diurnal patterns of photosynthesis, stomatal conductance, and microclimatic conditions.

Contrary to what many people had long assumed would be the case for a C<sub>4</sub> crop such as corn growing under the best of natural conditions, Leakey *et al.* found “growth at elevated CO<sub>2</sub> significantly increased leaf photosynthetic CO<sub>2</sub> uptake rate by up to 41%.” The highest whole-day increase was 21% (11 July) followed by 11% (22 July), during a period of low rainfall. Thereafter, however, during a period of greater rainfall, there were no significant differences between the photosynthetic rates of the plants in the two CO<sub>2</sub> treatments. Over the entire growing season, the CO<sub>2</sub>-induced increase in leaf photosynthesis averaged 10%.

Additionally, on all but the first day of measurements, stomatal conductance was significantly lower (–23% on average) in the elevated CO<sub>2</sub> treatment, which led to reduced transpiration rates in the CO<sub>2</sub>-enriched plants. And since “low soil water availability and high evaporative demand can both generate water stress and inhibit leaf net CO<sub>2</sub> assimilation in C<sub>4</sub> plants,” as Leakey *et al.* note, they conclude the lower transpiration rates of the plants growing in the CO<sub>2</sub>-enriched air “may have counteracted the development of water stress under elevated CO<sub>2</sub> and prevented the inhibition of leaf net CO<sub>2</sub> assimilation observed under ambient CO<sub>2</sub>.”

Therefore, they write, “contrary to expectations, this US Corn Belt summer climate appeared to cause sufficient water stress under ambient CO<sub>2</sub> to allow the ameliorating effects of elevated CO<sub>2</sub> to significantly enhance leaf net CO<sub>2</sub> assimilation.” They conclude this response of corn to elevated CO<sub>2</sub> “indicates the potential for greater future crop biomass and harvest-

able yield across the US Corn Belt,” due largely to the amelioration of water stress by CO<sub>2</sub>-induced decreases in transpirational water loss.

Grant *et al.* (2004) adjusted the crop growth and water relations model *ecosys* to represent the C<sub>4</sub> crop sorghum and ran it for two growing seasons (1 May 1998 to 31 October 1999) under both wet and dry irrigation regimes at two atmospheric CO<sub>2</sub> concentrations (approximately 368 and 561 ppm), using hourly meteorological data measured at a field south of Phoenix, Arizona (USA). They used these simulated energy balances and water relations—verified by measurements of energy flux and water potential—to infer the effects of free-air atmospheric CO<sub>2</sub> enrichment on various plant parameters and processes.

The 12 researchers report “model results, corroborated by field measurements, showed elevated CO<sub>2</sub> raised canopy water potential and lowered latent heat fluxes under high irrigation [both of which responses are beneficial] and delayed water stress under low irrigation [which is also beneficial].” As they described it elsewhere, the elevated CO<sub>2</sub> “reduced transpiration and hence improved water status of sorghum [and] lowered the vulnerability of sorghum CO<sub>2</sub> fixation to soil or atmospheric water deficits, even when irrigation was high.” Also, in applying their reality-tuned model to a scenario where the air’s CO<sub>2</sub> content was 50% higher and air temperature was 3°C greater, they calculated sorghum yields would rise by about 13%, and “current high sorghum yields could be achieved with ~120 mm or ~20% less irrigation water if these rises in temperature and CO<sub>2</sub> were to occur.”

The studies reviewed here suggest a continued rise of the atmosphere’s CO<sub>2</sub> content should allow production of substantially more food on each hectare of land with little or no change in per-hectare water requirements. In the case of C<sub>4</sub> crops, it appears higher yields may be produced with smaller amounts of water, even in the face of higher temperatures.

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### 2.31.1.2 Other Plants

- Most herbaceous non-crop plants respond to increases in the air’s CO<sub>2</sub> concentration by reducing their leaf stomatal conductances, which typically leads to reduced rates of evaporative water loss from them. The saved water, in turn, often leads to greater soil moisture content in CO<sub>2</sub>-enriched ecosystems, with positive effects on plant growth.

This section examines the results of some studies of C<sub>3</sub> and C<sub>4</sub> grassland species that broach various aspects of this multifaceted subject.

Szente *et al.* (1998) grew four perennial C<sub>3</sub> plants (two grasses and two broad-leaved species) common to loess grasslands of Budapest in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 or 700 ppm for 231 days. They found this doubling of the air’s CO<sub>2</sub> content significantly enhanced rates of net photosynthesis in all species studied, with the two grasses and the two broad-leaved species exhibiting average increases of 136 and 486%, respectively. The extra CO<sub>2</sub> increased transpiration rates for one of the grasses and one of the broad-leaved species, but it did not affect the water loss rates of the remaining species. Nevertheless, the two grasses exhibited an average CO<sub>2</sub>-induced increase in water use efficiency

of 72%, and the two broad-leaved species displayed an average increase of 366%. These large improvements should allow them to cope better with the hot and dry summers they characteristically experience in this part of Hungary.

Engloner *et al.* (2003) removed grassland monoliths with their original soils to a depth of 40 cm from a xeric loess grassland and relocated them to open-top chambers outside Budapest, where they were exposed for seven years to either ambient air or air enriched to a CO<sub>2</sub> concentration of 700 ppm. Measurements of leaf photosynthesis and transpiration rates of a dominant member of this ecosystem (couch-grass) were conducted throughout the experiment according to protocols described by Tuba *et al.* (1994, 1996), and measurements of starch and soluble sugars were made as described by Tuba *et al.* (1994). Rates of net photosynthesis increased by an average of 194% in response to the ~90% increase in atmospheric CO<sub>2</sub> concentration, leading to starch and soluble sugar increases of approximately 50 and 72%, respectively. Leaf transpiration rates declined by about 18%, leading to a 345% increase in water use efficiency, which for a xeric grassland species is like manna from heaven, greatly fortifying it against the rigors of its xeric environment.

Turning to C<sub>4</sub> plants, Seneweera *et al.* (1998) grew a drought-resistant perennial grass (*Panicum coloratum*) for five weeks in controlled environment chambers having atmospheric CO<sub>2</sub> concentrations of 350 or 1,000 ppm and different vapor pressure deficits (VPDs) maintained by keeping the relative humidity of the air at either 50 or 80%. The plants were watered daily to 65, 80, or 100% of their potting soils' field capacities. Under favorable environmental conditions, characterized by a low VPD and high soil moisture (100% field capacity), atmospheric CO<sub>2</sub> enrichment failed to cause any significant increases in leaf or stem dry weight. However, when water-stressed conditions prevailed, due to either a high VPD, low field capacities of 65 or 80%, or combinations of both parameters, elevated CO<sub>2</sub> caused large, significant increases in growth. At the high VPD, for example, the percentage increases in leaf dry weight at field capacities of 65 and 80% were 117 and 112%, respectively, and the growth responses for stems under these conditions were 50 and 57%.

These growth increases resulted in part from the ability of elevated CO<sub>2</sub> to ameliorate the negative effects of water stress on growth. Under the most extreme water-stressed condition, for example, leaf water potential values were about 3.5 times more negative, i.e., more stressful, for plants grown in air

of 350 ppm CO<sub>2</sub> than for plants grown in air of 1,000 ppm, because transpirational water loss was always less for plants grown in elevated CO<sub>2</sub>. For the most water-stressed condition investigated, which resulted from a high VPD and a field capacity of 65%, the transpiration rates of plants grown in ambient CO<sub>2</sub> were about 2.5 times greater than those of plants grown in elevated CO<sub>2</sub>. Consequently, higher concentrations of atmospheric CO<sub>2</sub> likely will allow C<sub>4</sub> grasses to maintain better internal water relations by reducing transpirational water losses, resulting in greater water-use efficiencies and the likely expansion of the plants into hot and arid regions commonly subjected to drought.

In an extension of this work, Seneweera *et al.* (2001) grew *P. coloratum* in controlled environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of 360 and 1,000 ppm for three weeks before withholding water from half the plants for 10 days, after which the plants were rewatered for five days to promote recovery. At the onset of water stress, shoot dry mass in the CO<sub>2</sub>-enriched plants was 33% greater than in the plants grown in ambient air. Although water stress reduced shoot dry mass, the reductions were less severe for CO<sub>2</sub>-enriched than ambiently grown plants. During the water stress treatment, leaf water potentials and leaf relative water contents dropped at much slower rates and to lesser degrees in the CO<sub>2</sub>-enriched plants than in the ambiently grown plants. Similarly, transpiration rates of the CO<sub>2</sub>-enriched plants were much less than those of plants growing in ambient air, and this phenomenon helped contribute to the greater soil moisture contents that were always present beneath the CO<sub>2</sub>-enriched plants. At final harvest, the CO<sub>2</sub>-induced enhancement of shoot dry mass was 44 and 70% for plants that had been subjected to well-watered and water-stressed treatments, respectively.

In a two-year experiment in an annual-dominated California grassland, Zavaleta *et al.* (2003) delivered extra heating to a number of FACE plots (enriched with an extra 300 ppm of CO<sub>2</sub>) via IR heat lamps that warmed the surface of the soil beneath them by 0.8–1.0°C. The individual effects of atmospheric CO<sub>2</sub> enrichment and soil warming were of similar magnitude, and acting together they enhanced mean spring soil moisture content by about 15% over that of the control plots. The effect of CO<sub>2</sub> was produced primarily as a consequence of its ability to cause partial stomatal closure and thereby reduce season-long plant water loss via transpiration. With warming, there was an acceleration of canopy senescence that further increased soil moisture by reducing the period

of time (the length of the growing season) over which transpiration losses occur, all without any decrease in total plant production.

The six researchers note their findings “illustrate the potential for organism-environment interactions to modify the direction as well as the magnitude of global change effects on ecosystem functioning.” Whereas for the past several years there have been multiple predictions of vast reaches of agricultural land drying up and being lost to profitable production in a CO<sub>2</sub>-enriched and warmed world of the future, this study suggests just the opposite could occur. As Zavaleta *et al.* describe it, “we suggest that in at least some ecosystems, declines in plant transpiration mediated by changes in phenology can offset direct increases in evaporative water losses under future warming.”

For both C<sub>3</sub> and C<sub>4</sub> grassland plants, the reductions in leaf transpirational water loss that result from increases in the air’s CO<sub>2</sub> concentration should significantly enhance their ability to withstand the rigors of periodic severe water stress in arid and semi-arid parts of the globe—even in the face of significant warming, which may induce its own beneficial feedback—while enhancing their productivities and thereby providing more forage for the various forms of animal life that inhabit these regions. In addition, these plants may reclaim great tracts of desert as their water use efficiencies rise to levels not experienced for millions of years.

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## 2.31.2 Woody Plants

As the air’s CO<sub>2</sub> content rises, the water use efficiencies (amount of carbon gain per unit of water loss) of coniferous trees and dryland shrubs tend to rise as well. The same is true of most deciduous trees, enabling them to cope more effectively with the increased water stress they would otherwise experience in stressful conditions and locations.

### 2.31.2.1 Conifers

- As the air’s CO<sub>2</sub> content rises, conifer water use efficiency (the amount of carbon gain at the needle level per unit of water loss) tends to rise as well.

Apple *et al.* (2000) grew two-year-old Douglas fir seedlings for three additional years in controlled environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 350 or 550 ppm and ambient or elevated (ambient plus 4°C) air temperature. They found the 200-ppm increase in the air’s CO<sub>2</sub> concentration and the 4°C increase in air temperature, when applied together, did not significantly affect seedling transpiration. When applied alone, however, the extra CO<sub>2</sub> reduced transpiration by 12%, and the elevated air temperature increased it by 66%.

After a one-year continuation of the same experiment, Lewis *et al.* (2002) report results obtained over its final 21 months. Once again, the extra CO<sub>2</sub> decreased transpiration by 12%, but the elevated air temperature increased it by 37%, considerably less than the 66% increase report by Apple *et al.* Adding more confusion, the combination of the identical CO<sub>2</sub> effect and a weaker temperature

effect yielded a 19% increase in transpiration, whereas in the first three years of the study the identical CO<sub>2</sub> effect and a stronger temperature effect produced no change in transpiration.

In a one-year study in which closed-top chambers were constructed around 30-year-old Scots pine trees growing in Finland, Kellomaki and Wang (1998) applied doubled CO<sub>2</sub> to half the trees while studying the effect of a 4°C increase in air temperature. The elevated CO<sub>2</sub> reduced cumulative sap flow (a measure of transpiration) by 14% in the trees maintained at ambient temperatures, but when both air temperature and CO<sub>2</sub> concentration were increased together, cumulative sap flow exhibited no change.

In the studies cited above, atmospheric CO<sub>2</sub> enrichment consistently produced a small reduction in tree transpiration rates at ambient air temperatures, and the added effect of warming was inconsistent and confusing. Hence, it is instructive to consider the study of Saurer *et al.* (2004), who measured carbon isotope ratios in the rings of coniferous trees of northern Eurasia (including the genera *Larix*, *Picea* and *Pinus*) across a longitudinal transect that covered the entire supercontinent in the latitude range 59–71°N. Between the two 30-year periods 1861–1890 and 1961–1990, when air temperature and CO<sub>2</sub> concentration both rose significantly, they found intrinsic water use efficiency (Wi, the amount of carbon gain at the needle level per unit of water loss) rose significantly, such that “125 out of 126 trees showed increasing Wi from 1861–1890 to 1961–1990, with an average improvement of 19.2 ± 0.9%.”

The three Swiss scientists say their results suggest the trees they studied “are able to produce the same biomass today [as they did 100 years ago] but with lower costs in terms of transpiration.” This finding is very important because, they write, recent warming in other longitudinal segments of the same latitude belt “may be accompanied by increased drought stress (Lloyd and Fastie, 2002)” and the ongoing rise in the air’s CO<sub>2</sub> content may be helping the trees in those areas to better cope with this environmental challenge, even in the face of increasing air temperatures.

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### 2.31.2.2 Dryland Plants

- As the air’s CO<sub>2</sub> content rises, the water-use efficiencies of Earth’s dryland plants usually tend to rise as well.

In a FACE experiment conducted in a chaparral region of southern California, Roberts *et al.* (1998) exposed *Adenostoma fasciculatum* shrubs to atmospheric CO<sub>2</sub> concentrations of 360 and 550 ppm. After six months of treatment, it was clear the elevated CO<sub>2</sub> had reduced the stomatal conductances of the shrubs’ leaves and decreased their rates of evaporative water loss. The CO<sub>2</sub>-enriched shrubs exhibited leaf water potentials significantly more positive (less stressful) than those of the plants growing in ambient air. This enhancement of internal water status should help this woody perennial better withstand the periods of drought that commonly occur throughout its southern California range.

Dugas *et al.* (2001) studied the response of whole-plant transpiration to atmospheric CO<sub>2</sub> enrichment in the woody legume *Acacia farnesiana*, which occurs throughout the south-central United States and is one of the most aggressive woody-plant invaders of grasslands worldwide. Plants of this species were grown for a full year in greenhouse bays continuously maintained at atmospheric CO<sub>2</sub> concentrations of either 385 or 980 ppm, and at the end of this time whole-plant transpiration was assessed via sap flow measurements. The mean transpiration rate of the plants grown at an atmospheric CO<sub>2</sub> concentration of 980 ppm was only about one-fourth of that exhibited by the plants grown at a concentration of 385 ppm.

The increase in water use efficiency implied by this result could explain *A. farnesiana*'s increasing ascendancy over dryland grasses as the air's CO<sub>2</sub> content has risen over the past century or more.

Peterson and Neofotis (2004) sprouted and grew velvet mesquite plants in small pots within controlled environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 380 or 760 ppm. Although they did not see a large CO<sub>2</sub>-induced increase in plant growth, by the end of their six-week study there was a highly significant 41% reduction in the volume of water transpired by the mesquite seedlings in the CO<sub>2</sub>-enriched treatment. "This large reduction in whole-plant water use," they write, "occurred because the reduction in transpiration per unit leaf area at elevated CO<sub>2</sub> was not offset by a proportional increase in total leaf area."

The pair of scientists from the Biosphere 2 Center near Oracle, Arizona, state their findings suggest in a future high-CO<sub>2</sub> world, mesquite seedlings "may deplete soil moisture at a slower rate than they do currently," and "this could facilitate seedling survival between intermittent rain events." They also note their work "corroborates the conclusions of Polley *et al.* (1994, 1999, 2003) that increasing levels of atmospheric CO<sub>2</sub> may facilitate the establishment of mesquite seedlings through a reduction in soil water depletion." Peterson and Neofotis note "mesquites and other woody species in the semiarid southwestern United States have shown substantial increases in population density and geographic range since Anglo-American settlement of the region approximately 120 years ago," citing Van Auken and Bush (1990), Gibbens *et al.* (1992), Bahre and Shelton (1993), Archer (1995), Boutton *et al.* (1999), Van Auken (2000), Ansley *et al.* (2001), Wilson *et al.* (2001), and Biggs *et al.* (2002).

Just as nonagricultural herbaceous plants are encroaching on Earth's deserts as the air's CO<sub>2</sub> content rises, so too are woody dryland shrubs following in their "rootsteps," as the greening of Earth continues.

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### 2.31.2.3 Deciduous Trees

- Most deciduous trees exhibit modest reductions in transpirational water loss in CO<sub>2</sub>-enriched air, enabling them to cope more effectively with the increased water stress they otherwise would experience under water-limiting conditions.

Wayne *et al.* (1998) grew yellow birch seedlings in controlled environment chambers maintained at atmospheric CO<sub>2</sub> concentrations of either 400 or 800 ppm and day/night air temperatures of either 26/21 or 31/26°C for two months. They determined the elevated CO<sub>2</sub> treatment stimulated net photosynthesis by 48% at both temperatures, and it increased seedling biomass by 60% and 227% at normal and high air temperatures, respectively. In addition, because the extra CO<sub>2</sub> reduced transpiration by 25 and 36% at the normal and high air temperatures, plant water use efficiency rose by 52 and 94% in these two situations, which suggests yellow birch may someday be able to expand into regions where high summer temperatures and limited rainfall currently discourage its presence.

In a FACE study established within a 10-year-old stand of sweetgum trees growing on a nutrient-rich soil in Tennessee (USA), Wullschleger and Norby (2001) measured CO<sub>2</sub>-induced sap-flow reductions that averaged 13% over the growing season. The elevated CO<sub>2</sub> (540 vs. 390 ppm) also reduced growing-season transpiration rates by about the same amount, leading to a 28% increase in stand-level water use efficiency in the CO<sub>2</sub>-enriched trees. In a second year of measurements, Wullschleger *et al.* (2002) found the CO<sub>2</sub>-enriched air reduced the stomatal conductances of individual leaves by an average of 23% across the growing season. When extrapolated to the entire canopy, however, the reduction fell to 14%, and there was a 7% reduction in stand evapotranspiration.

Tognetti *et al.* (1998) studied the effects of naturally occurring elevated CO<sub>2</sub> concentrations (500 to 1,000 ppm) near a CO<sub>2</sub>-emitting spring in central Italy on summer water relations of mature oak trees by measuring leaf stomatal conductances and trunk sap flow rates (a measure of transpiration) over a period of two years and comparing the results with

those obtained from similar-age oaks (15 to 25 years old) growing in air of ambient CO<sub>2</sub> concentration at a site about three kilometers away. As both summers were characterized by severe drought, rates of water loss were relatively high in both sets of trees. Leaf stomatal conductances, however, were significantly lower in the trees growing near the CO<sub>2</sub> springs, as were trunk sap velocities. These findings suggest the trees near the CO<sub>2</sub>-emitting springs experienced less water loss and maintained a more favorable internal water status than those growing in non-CO<sub>2</sub>-enriched ambient air.

Tognetti *et al.* also determined the trees growing near the CO<sub>2</sub>-emitting springs possessed less foliage area than the control trees, and this reduction in transpirational surface area allowed the CO<sub>2</sub>-enriched trees to maintain a better internal water status than the control trees during periods of drought. The researchers state the reduction in foliage area was “equally, if not more, effective than stomatal closure in reducing transpiration and plant water use under elevated CO<sub>2</sub>.” Thus if drought situations continue to recur during future Italian summers, the rising CO<sub>2</sub> content of the atmosphere should provide oak, and perhaps other trees, with at least two different mechanisms for sustaining their growth during protracted periods of reduced water availability.

In a more broad-ranging study, Cech *et al.* (2003) enriched the air’s CO<sub>2</sub> content within the canopy of a 30-meter-tall species-rich forest just south of Basel, Switzerland, using the web-FACE technique of Pepin and Korner (2002), to a mean value of 520 ppm for an entire growing season to test, they write, “whether elevated CO<sub>2</sub> reduces water use in mature forest trees.” They took sap flow measurements of 14 broadleaved trees (three *Fagus*, four *Quercus*, four *Carpinus*, one *Tilia*, one *Acer*, and one *Prunus*) and their ambient-treatment counterparts via the constant heat-flow technique of Granier (1985, 1987) from 5 June to 1 October 2001.

Over the growing season, the extra 150 ppm of CO<sub>2</sub> reduced mean daily sap flow across all species by an average of 10.7%. At one end of the spectrum, the reductions were high (22%) when the evaporative demand of the air was low (mean daily vapor pressure deficits less than 5 hPa); but they were small (2%) at the other end, when the evaporative demand of the air was high (mean daily vapor pressure deficits greater than 10 hPa). The researchers conclude the “daily water savings by CO<sub>2</sub>-enriched trees may have accumulated to [produce] a significantly improved water status by the time when control trees were short of soil moisture,” so “CO<sub>2</sub>-enriched trees would enter

drier periods with a higher soil moisture capital, permitting prolonged gas exchange (for a few days).”

In light of these several experimental observations, it would appear most deciduous trees exhibit modest reductions in transpirational water loss in CO<sub>2</sub>-enriched air. Consequently, if the planet continues to warm and experience increased dryness in some regions, Earth’s deciduous trees may more effectively deal with the increased water stress they otherwise would experience in these places, if the air’s CO<sub>2</sub> content continues to rise as well.

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- Maier *et al.* (2008) described how a soil nitrogen fertilizer application affected upper-canopy needle morphology and gas exchange in 20-meter-tall loblolly pine (*Pinus taeda* L.) trees previously exposed to elevated atmospheric CO<sub>2</sub> concentrations (200 ppm above ambient) for nine years at the Duke Forest FACE facility in Orange County, North Carolina (USA). During the tenth year of exposure to elevated CO<sub>2</sub>, there was a strong enhancement (greater than 50%) of light-saturated net photosynthesis across all age classes of needles, and the stimulation was 28% greater in current-year foliage than in one-year-old foliage. In addition, current-year foliage incorporated the added nitrogen into photosynthetic components that increased the photosynthetic capacity of the current-year foliage, but the one-year-old foliage tended to simply store extra nitrogen, which subsequently served as “an important source of nitrogen for the development of current-year foliage” via “efficient retranslocation of nitrogen from senescing one-year-old foliage to developing foliage.”
- These findings echo those observed several years earlier in sour orange tree (*Citrus aurantium* L.) foliage in an open-top chamber experiment conducted at Phoenix, Arizona (USA) by Idso *et al.* (2001), where half the trees they studied had been grown from the seedling stage for the prior six years in air continuously enriched with an extra 300 ppm of CO<sub>2</sub>. In the seventh year of that study, the Arizona researchers identified three putative vegetative storage proteins located within amorphous material in the vacuoles of leaf mesophyll cells that was rerouted, “starting at about day 25 of the new year, into developing foliage on the new branch buds of the CO<sub>2</sub>-enriched trees.” They speculate this phenomenon may have been “the key that allows the CO<sub>2</sub>-enriched trees to temporarily stockpile the unusually large pool of nitrogen that is needed to support the large CO<sub>2</sub>-induced increase in new-branch growth that is observed in the spring,” citing Idso *et al.* (2000), who previously found 24 days after new-branch emergence in the spring, “the new branches of the CO<sub>2</sub>-enriched trees were, on average, 4.4 times more massive than the new branches of the trees growing in ambient air,” and “the total new-branch tissue produced on the CO<sub>2</sub>-enriched trees to that point in time was over six times greater than that produced on

## 2.32 Vegetative Storage Proteins

- Atmospheric CO<sub>2</sub> enrichment appears to enhance the between-cuttings savings of vegetative storage proteins (VSPs) in the roots of alfalfa crops, as well as the between-years storage of

the ambient-treatment trees.”

If there is a common mechanism that links the results of the two groups, it could revolve around the hypothesized vacuolar storage proteins Idso *et al.* (2001) identified in the sour orange tree foliage, since they detected immunologically related proteins in a variety of other citrus species, but not in 20 different grasses, shrubs, and trees growing in the Biosphere 2 facility near Oracle, Arizona. This possibility merits further study because if it is found to have merit, Idso *et al.* (2001) further speculate the proteins in question “could possibly be genetically exploited to enhance the responses of other plant species to atmospheric CO<sub>2</sub> enrichment.” This could prove to be a valuable property of agriculturally important plants in a high-CO<sub>2</sub> world of the future.

Erice *et al.* (2007) studied 30-day-old nodulated alfalfa (*Medicago sativa* L.) plants grown in two temperature-gradient greenhouses (one maintained at an atmospheric CO<sub>2</sub> concentration of 350 ppm and the other at a concentration of 700 ppm) in pots recessed into the ground in an alfalfa field under conditions of ambient temperature (TA) and elevated temperature (TE = TA + 4°C) and well-watered (to field capacity) and water-stressed (50% field capacity) conditions for one month, after which a first cutting took place, and for one additional month, after which a second cutting took place. After each cutting, the scientists determined plant dry matter production and analyzed taproots for vegetative storage protein contents.

At the time of first cutting, the alfalfa plants had had their dry matter production boosted by an average of about 30% in the well-watered treatment (averaged across both temperature treatments) over the first growth period, but by only about 10% in the water-stressed treatment. At the time of the second cutting, however, the well-watered plants had experienced an average dry matter production increase on the order of 20% over the second growth period, and the plants in the water-stressed treatment displayed a mean increase of fully 40%. In addition, Erice *et al.* report over the first growth period “taproot vegetative storage protein content increased in response to drought and elevated CO<sub>2</sub>.”

The researchers thus state, “it has been demonstrated that nitrogen pools in alfalfa taproot, especially vegetative storage proteins, condition new regrowing shoots,” and that appears to be what happened in their study. At the end of the first growth period, for example, the enhanced taproot vegetative storage protein content in the water-stressed and CO<sub>2</sub>-enriched treatment may have been the reason the elevated CO<sub>2</sub> was so effective in stimulating biomass

production in the water-stressed treatment over the second growth period.

This finding is somewhat analogous to the observation of Idso *et al.* (2001), who found nitrogen reabsorbed from second-year leaves of sour orange trees (which hold most of their leaves for two years) during the process of senescence in the fall was stored over winter in much greater amounts in putative vegetative storage proteins in first-year leaves of CO<sub>2</sub>-enriched trees than in first-year leaves of trees growing in ambient air. When the stored nitrogen was released in the spring to produce a flush of new leaves on the trees, leaf production on the CO<sub>2</sub>-enriched trees vastly outpaced the production of new leaves on trees growing in ambient air. Taken together, these observations indicate yet another of the manifold benefits of atmospheric CO<sub>2</sub> enrichment.

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## 2.33 Water Use Efficiency

As the atmosphere’s CO<sub>2</sub> concentration rises, most plants tend to exhibit increased rates of net photosynthesis and biomass production, and on a per-unit-leaf-area basis, plants exposed to elevated CO<sub>2</sub> concentrations are likely to lose less water via transpiration because they tend to exhibit lower stomatal conductances. Therefore, the amount of carbon gained per unit of water lost per unit leaf

area—or water use efficiency—should increase significantly as the air's CO<sub>2</sub> content rises. The following three sub-sections present evidence for this phenomenon in agricultural crops, grasslands, and trees.

### 2.33.1 Agricultural Species

- As the CO<sub>2</sub> content of the air continues to rise, nearly all agricultural plants will respond favorably by exhibiting increases in water use efficiency. It is also thus likely food and fiber production will increase on a worldwide basis, even in areas where productivity is currently severely restricted by limited availability of soil moisture.

Serraj *et al.* (1999) found soybeans grown at 700 ppm CO<sub>2</sub> displayed 10 to 25% reductions in total water loss while simultaneously exhibiting dry weight increases of as much as 33%. Thus, elevated CO<sub>2</sub> significantly increased the water-use efficiencies of the plants. Likewise, Garcia *et al.* (1998) determined spring wheat grown at 550 ppm CO<sub>2</sub> exhibited a water use efficiency about one-third greater than that of plants grown at 370 ppm CO<sub>2</sub>. Similarly, Hakala *et al.* (1999) report twice-ambient CO<sub>2</sub> concentrations increased the water use efficiency of spring wheat by 70 to 100%, depending on experimental air temperature. In addition, Hunsaker *et al.* (2000) report CO<sub>2</sub>-induced increases in water use efficiency for field-grown wheat 20 and 10% higher than those displayed by ambiently grown wheat subjected to high and low soil nitrogen regimes, respectively. Also, pea plants grown for two months in growth chambers maintained at CO<sub>2</sub> concentrations of 700 ppm displayed an average water use efficiency 27% greater than that exhibited by ambiently grown control plants (Gavito *et al.*, 2000).

In some cases, the water use efficiency increases caused by atmospheric CO<sub>2</sub> enrichment are spectacularly high. De Luis *et al.* (1999), for example, demonstrated alfalfa plants subjected to atmospheric CO<sub>2</sub> concentrations of 700 ppm had water-use efficiencies 2.6 and 4.1 times greater than those of control plants growing at 400 ppm CO<sub>2</sub> under water-stressed and well-watered conditions, respectively. Also, when grown at an atmospheric CO<sub>2</sub> concentration of 700 ppm, a 2.7-fold increase in water use efficiency was reported by Malmstrom and Field (1997) for oats infected with the barley yellow dwarf virus.

In addition to enhancing the water-use efficiencies of agricultural C<sub>3</sub> crops, elevated CO<sub>2</sub> also enhances the water-use efficiencies of crops possessing alternate carbon fixation pathways. Maroco *et al.* (1999), for example, demonstrated maize—a C<sub>4</sub> crop—grown for 30 days at an atmospheric CO<sub>2</sub> concentration of 1,100 ppm exhibited an intrinsic water use efficiency 225% higher than that of plants grown at 350 ppm CO<sub>2</sub>. In addition, Conley *et al.* (2001) report a 200-ppm increase in the air's CO<sub>2</sub> content boosted the water use efficiency of field-grown sorghum by 9 and 19% under well-watered and water-stressed conditions, respectively. Also, Zhu *et al.* (1999) report pineapple—a CAM plant—grown at 700 ppm CO<sub>2</sub> exhibited water-use efficiencies always significantly greater than those of control plants grown at 350 ppm CO<sub>2</sub> over a wide range of growth temperatures.

Olivo *et al.* (2002) grew two potato species (*Solanum curtilobum* cv. Ugro Shiri, from high altitude, and *S. tuberosum* cv. Baronesa, from low altitude) in pots placed within open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm for 30 days following the onset of reproductive growth, to study the effects of elevated CO<sub>2</sub> on gas exchange and biomass production in these two species in the first-ever study of the CO<sub>2</sub> responsiveness of the high-altitude-adapted *Solanum curtilobum*, which is economically important in the highlands of the South American Andes. The elevated CO<sub>2</sub> treatment increased rates of net photosynthesis by 56 and 53% in the high- and low-altitude potato species, respectively, while reducing their stomatal conductances by 55 and 59% and thereby increasing their water-use efficiencies by 90 and 80%, respectively. Tuber dry mass production was increased by 85 and 40% in the high- and low-altitude potato species, respectively.

Dong-Xiu *et al.* (2002) grew spring wheat (*Triticum aestivum* L. cv. Gaoyuan 602) in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm and three levels of soil moisture (40, 60, and 80% of field capacity), to study the interactive effects of these environmental variables on the productivity and growth of this variety of wheat. The elevated CO<sub>2</sub> treatment increased rates of net photosynthesis by 48, 120, and 97% at low, medium, and high soil water capacities, respectively, and it reduced rates of transpiration by 56, 53, and 63% in the same order. These changes led to CO<sub>2</sub>-induced increases in plant water use efficiency of approximately 25, 15, and 30% under low, medium, and high soil moisture conditions,

respectively.

Agrawal and Deepak (2003) grew two other cultivars of wheat (*Triticum aestivum* L. cv. Malviya 234 and HP1209) in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 350 and 600 ppm alone and in combination with 60 ppb of sulfur dioxide (SO<sub>2</sub>), to study the interactive effects of elevated CO<sub>2</sub> and this major air pollutant on the growth and yield of this important crop. They found exposure to elevated CO<sub>2</sub> significantly increased photosynthetic rates by 58 and 48% in M234 and HP1209, respectively, and fumigation with elevated SO<sub>2</sub> did not significantly impact rates of photosynthesis in either cultivar. However, plants grown in the combined treatment of elevated CO<sub>2</sub> and elevated SO<sub>2</sub> displayed photosynthetic rates significantly less-enhanced to values of 42 and 38% greater than those measured in control plants for M234 and HP1209, respectively.

Of more significance to the topic of this review, however, plants grown in elevated CO<sub>2</sub> also displayed an approximate 20% reduction in stomatal conductance, and those grown in elevated SO<sub>2</sub> exhibited an average increase of 15%. But when exposed simultaneously to both gases, the plants displayed an average 11% reduction in stomatal conductance, which resulted in a 32% increase in water use efficiency, whereas plants exposed to elevated SO<sub>2</sub> alone displayed an average decrease in water use efficiency of 16%.

Working with a very different type of crop, Kyei-Boahen *et al.* (2003) grew well-watered and well-fertilized plants of four carrot (*Daucus carota* var. sativus L.) cultivars (Cascade, Caro Choice, Oranza, and Red Core Chantenay) from seed in 15-cm-diameter plastic pots in a controlled environment facility for 30 days past emergence. Leaf net photosynthetic rate (PN), stomatal conductance (Gs), and transpiration rate (E) were then measured at 100-ppm intervals of short-term (five-minute) atmospheric CO<sub>2</sub> enrichment yielding absolute CO<sub>2</sub> concentrations (Ca) stretching from 50 to 1,050 ppm. The five researchers found “an increase in Ca from 50 to 350 ppm produced a 100-fold increase in PN and the value increased by 43% when Ca was elevated from 350 to 650 ppm,” but “only [a] 7% increase in PN was observed when Ca was increased from 650 to 1050 ppm.”

They also found “increasing Ca from 50 to 350 ppm increased Gs to a maximum and thereafter Gs declined by 17% when Ca was increased to 650 ppm,” and “a three-fold increase in Ca from 350 to 1050 ppm decreased Gs by 53%.” They further

report, “E reached maximum values (0.9–1.1 mmol m<sup>-2</sup> s<sup>-1</sup>) at 350 ppm followed by a decline to 0.40–0.60 mmol m<sup>-2</sup> s<sup>-1</sup> when Ca was increased to 1050 ppm.” Finally, “water use efficiency increased linearly with Ca due to increases in PN in addition to the decline in E at high Ca,” so “increasing Ca from 350 to 650 ppm improved water use efficiency by 76%, whereas a three-fold increase in Ca from 350–1050 ppm resulted in a three-fold increase in water use efficiency” They conclude, “future enrichment in the atmospheric CO<sub>2</sub> may lead to adjustments in PN and Gs, which could improve carrot productivity and water utilization.”

Triggs *et al.* (2004) grew sorghum (*Sorghum bicolor* (L.) Moench, a C<sub>4</sub> grain crop) for two full seasons in control CO<sub>2</sub> plots (about 370 ppm) and FACE plots (Control + 200 ppm) under both well-watered (Wet) and water-stressed (Dry, less than half the total water received by the Wet treatment via rainfall and irrigation) conditions near Maricopa, Arizona (USA), assessing evapotranspiration (ET) on a continuous basis by means of micrometeorological measurements designed to allow the calculation of all of the other elements (net radiation, sensible heat flux, and soil surface heat flux) of the energy balance of the crop-soil interface with the atmosphere.

Triggs *et al.* report, “In the Wet treatments, a reduction in ET of about 19%, combined with only a slight increase in total biomass (+4%), resulted in a 28% increase in WUE in elevated CO<sub>2</sub> conditions,” and “in the Dry treatments, the relatively large increase in total biomass (+16% for both years) more than compensated for the approximate 5% increase in total ET, giving the FACE-Dry treatments an increase in WUE of 16% over both seasons.” Based on these results, Triggs *et al.* conclude, “even if future climate change results in less water available for agriculture, higher atmospheric CO<sub>2</sub> concentrations will still benefit C<sub>4</sub> crops,” but they note, “in regions with ample precipitation or irrigation, C<sub>3</sub> crops with higher growth responses may be preferable.”

Grant *et al.* (2004) adjusted the crop growth and water relations model *ecosys* to represent sorghum (*Sorghum bicolor* (L.) Moench) and run for two growing seasons (1 May 1998 to 31 October 1999) under both wet and dry irrigation schedules at two atmospheric CO<sub>2</sub> concentrations (approximately 368 and 561 ppm) using hourly meteorological data measured at a field south of Phoenix, Arizona (USA), after which the crop’s simulated energy balances and water relations—verified by measurements of energy flux and water potential—were used to infer the effects of free-air atmospheric CO<sub>2</sub> enrichment on various plant parameters and processes.

The 12 researchers write, “model results, corroborated by field measurements, showed that elevated CO<sub>2</sub> raised canopy water potential and lowered latent heat fluxes under high irrigation [both of which responses are beneficial] and delayed water stress under low irrigation [which is also beneficial],” such that the elevated CO<sub>2</sub> “reduced transpiration and hence improved water status of sorghum [and] lowered the vulnerability of sorghum CO<sub>2</sub> fixation to soil or atmospheric water deficits, even when irrigation was high.” In applying their reality-tuned model to a scenario where the air’s CO<sub>2</sub> content was 50% higher and air temperature was 3°C greater, they calculated that sorghum yields would rise by about 13%, and “current high sorghum yields could be achieved with ~120 mm or ~20% less irrigation water if these rises in temperature and CO<sub>2</sub> were to occur.” Their real-world data and their analysis of those data indicate rising atmospheric CO<sub>2</sub> concentrations, even in the face of rising air temperatures, should be good for both sorghum and the people who grow it, in terms of both the higher yields that can be produced under these conditions and the smaller amounts of water required to produce them.

Yoshimoto *et al.* (2005) grew rice (*Oryza sativa* L. cv. Akita-Komachi) from hand-transplanting to harvest (May to September) under normal paddy culture near Shizukuishi, Iwate (Japan) within FACE rings maintained at either ambient or ambient + 200 ppm CO<sub>2</sub> for 24 hours per day. Over this period they measured a number of micrometeorological parameters and plant characteristics that enabled them to calculate both the amount of water lost directly from the paddy-water surface and that lost by plant transpiration, which together with the plant biomass data they obtained at harvest enabled them to calculate total growing-season crop water use efficiency.

Yoshimoto *et al.* determined “elevated CO<sub>2</sub> reduced stomatal conductance by 13% in upper leaves and by 40% in lower leaves at the panicle initiation stage,” but the reduction declined thereafter. In addition, “stomata closed more in the elevated CO<sub>2</sub> plot as vapor pressure deficit increased,” i.e., during drier conditions. In more common terms, and averaged over the entire growing season, the Japanese researchers determined the total water used by the crop was 268.7 mm in the ambient CO<sub>2</sub> treatment and 246.7 mm in the elevated CO<sub>2</sub> treatment. Combining this CO<sub>2</sub>-induced reduction in total evaporative water loss (8.2%) with the CO<sub>2</sub>-induced increase in total plant biomass that was observed (9.1%) indicated season-long crop water use efficiency rose by about

19% in response to the approximate 54% increase in atmospheric CO<sub>2</sub> concentration provided by the FACE apparatus. Thus, as world population continues to grow, the increase in rice-crop water use efficiency provided by the concomitant increase in the atmosphere’s CO<sub>2</sub> concentration should be a great asset in helping to produce the extra food that will be needed to feed the planet’s many newcomers, while sparing some of the precious water that will be needed to slake their thirst.

Kim *et al.* (2006) grew well-watered and fertilized maize (*Zea mays* L. cv. Pioneer 3733) plants from seed to developmental stage R3 (milky ripe stage, 70 days after planting) in sunlit soil-plant-atmosphere research (SPAR) chambers maintained at either 370 (ambient) or 750 (elevated) ppm CO<sub>2</sub> concentrations, while periodically measuring a number of plant physiological parameters. They discovered that at saturating photosynthetically active radiation (PAR, 2000 μmol m<sup>-2</sup> s<sup>-1</sup>), rates of leaf net photosynthesis in the elevated CO<sub>2</sub> chambers were only 4% greater than those in the ambient CO<sub>2</sub> chambers. However, upper-canopy leaves grown at elevated CO<sub>2</sub> exhibited reductions in excess of 50% in both leaf stomatal conductance and transpiration, and as a result of these changes, instantaneous leaf water use efficiency was more than doubled in the high-CO<sub>2</sub> treatment. Because of shading within the canopy, and because the difference in stomatal conductance between ambient and elevated CO<sub>2</sub> declines as PAR drops, the CO<sub>2</sub>-induced reduction in canopy evapotranspiration was only a little over 20%.

Commenting on their findings, the seven scientists write, “although several previous studies report CO<sub>2</sub> enrichment enhanced the growth of maize under well watered and fertilized conditions,” in their study “there was little evidence of increased biomass accumulation.” On the other hand, they state the sizable CO<sub>2</sub>-induced reduction in canopy evapotranspiration “is comparable to findings from other studies of C<sub>4</sub> plants.” Although the maize plants of their study were somewhat anomalous in not undergoing a significant boost in growth in response to atmospheric CO<sub>2</sub> enrichment, their more characteristic transpiration response enabled them to produce their slightly enhanced biomass with a considerably smaller consumption of water than that of the plants growing in ambient air.

Cunniff *et al.* (2008) note, “early agriculture was characterized by sets of primary domesticates or ‘founder crops’ that were adopted in several independent centers of origin,” all at about the same time; and they speculate, “this synchronicity suggests

the involvement of a global trigger.” Further noting Sage (1995) saw a causal link between this development and the rise in atmospheric CO<sub>2</sub> concentration that followed deglaciation (a jump from about 180 to 270 ppm), they hypothesize the aerial fertilization effect caused by the rise in CO<sub>2</sub> combined with its transpiration-reducing effect led to a large increase in the water use efficiencies of the world’s major C<sub>4</sub> founder crops, and this development was the global trigger that launched the agricultural enterprise. To test this hypothesis, they designed “a controlled environment experiment using five modern day representatives of wild C<sub>4</sub> crop progenitors, all ‘founder crops’ from a variety of independent centers.”

The five crops employed in their study were *Setaria viridis* (L.) P. Beauv., *Panicum miliaceum* var. *ruderales* (Kitag.), *Pennisetum violaceum* (Lam.) Rich., *Sorghum arundinaceum* (Desv.), and *Zea mays* subsp. *parviglumis* H.H. Iltis & Doebley. Each was grown individually in 6-cm x 6-cm x 6-cm pots filled with a 1:1 mix of washed sand and vermiculite for 40–50 days within growth chambers maintained at atmospheric CO<sub>2</sub> concentrations of 180, 280, and 380 ppm, characteristic of glacial, post-glacial, and modern times, respectively. The scientists found the “increase in CO<sub>2</sub> from glacial to postglacial levels [180 to 280 ppm] caused a significant gain in vegetative biomass of up to 40%,” together with “a reduction in the transpiration rate via decreases in stomatal conductance of ~35%,” which led to “a 70% increase in water use efficiency, and a much greater productivity potential in water-limited conditions.”

The five researchers conclude, “these key physiological changes could have greatly enhanced the productivity of wild crop progenitors after deglaciation ... improving the productivity and survival of these wild C<sub>4</sub> crop progenitors in early agricultural systems.” They note, “the lowered water requirements of C<sub>4</sub> crop progenitors under increased CO<sub>2</sub> would have been particularly beneficial in the arid climatic regions where these plants were domesticated.”

For comparative purposes, the researchers also had included one C<sub>3</sub> species in their study—*Hordeum spontaneum* K. Koch—and they report it “showed a near-doubling in biomass compared with [the] 40% increase in the C<sub>4</sub> species under growth treatments equivalent to the postglacial CO<sub>2</sub> rise.”

The civilizations of the past, which could not have existed without agriculture, were largely made possible by the increase in the air’s CO<sub>2</sub> content that accompanied deglaciation. The peoples of Earth today

are likewise indebted to this phenomenon, as well as to the additional 100 ppm of CO<sub>2</sub> the atmosphere has subsequently acquired. The ongoing rise in the air’s CO<sub>2</sub> content is likely to play a pivotal role in enabling society to grow the food needed to sustain the still-expanding global population without usurping all the planet’s remaining freshwater resources and much of its untapped arable land.

Morison *et al.* (2007) discuss at length the water use challenge, noting “agriculture accounts for 80–90% of all freshwater used by humans,” “most of that is in crop production,” and “in many areas, this water use is unsustainable.” They also note “farmers in many countries are now faced with legislative restrictions on use of water,” indicating the Chinese government has “set a target of a reduction of 20% in water use in agriculture by the year 2020,” but “if food security for the region is not to be threatened, this must be achieved without a loss in production.” How is this global food and water crisis to be overcome?

In their many pages of discussion of the subject, the four UK researchers examine underlying relationships that connect crop carbon uptake, growth, and water loss, noting “much effort is being made to reduce water use by crops and produce ‘more crop per drop.’” Some of the topics they examine in the course of this discussion were designed to alter various crop characteristics that might increase their water use efficiency, such as genetic engineering, and others deal with crop management strategies, such as how and when to apply irrigation water. As reported by the studies discussed above, and additional studies discussed below, water use efficiencies driven by increasing levels of atmospheric CO<sub>2</sub> will help address the problem.

Fleisher *et al.* (2008) grew potato (*Solanum tuberosum* cv. Kennebec) plants from “seed tubers” in soil-plant-atmosphere research (SPAR) chambers maintained at daytime atmospheric CO<sub>2</sub> concentrations of either 370 or 740 ppm in a 75/25 mix of coarse sand and vermiculite at well-watered and progressively water-stressed conditions until they were harvested when canopy photosynthetic rates dropped to below 50% of their seasonal peak values, before and after which they measured a variety of plant physical properties and physiological parameters.

They write, “elevated CO<sub>2</sub> plants maintained a higher daily net assimilation rate throughout most of the growing season,” and “at moderate and more severe levels of water stress, CO<sub>2</sub> enrichment appeared to encourage shifting of assimilate into

tubers as opposed to additional vegetative growth.” Hence, “total biomass, yield and water use efficiency increased under elevated CO<sub>2</sub>, with the largest percent increases occurring at irrigations that induced the most water stress,” and “water use efficiency was nearly doubled under enriched CO<sub>2</sub> when expressed on a tuber fresh weight basis.” Overall, “the results indicate that increases in potato gas exchange, dry matter production and yield with elevated CO<sub>2</sub> are consistent at various levels of water stress as compared with ambient CO<sub>2</sub>.”

Ceusters *et al.* (2008) measured gas exchange and diel metabolite (e.g. malate, soluble sugars, starch) dynamics in the youngest fully expanded leaves of well-watered and well-fertilized CAM bromeliad *Aechmea* ‘Maya’ plants—a spineless cultivar resulting from a cross between *A. tessmannii* and *A. fasciata*—after exposure of half of the original seven-month-old plants to 700 ppm CO<sub>2</sub> for five more months in one of two controlled-environment compartments of a greenhouse, and the other compartment was maintained at the original atmospheric CO<sub>2</sub> concentration of 380 ppm. They report, “there was a 60% increase in 24-hour carbon gain under elevated CO<sub>2</sub> due to a stimulation of daytime C<sub>3</sub> and C<sub>4</sub> carboxylation,” and they note water use efficiency was twofold higher during the night under elevated CO<sub>2</sub> and three- to four-fold higher during the day.

The six scientists state the great increase they observed in plant water use efficiency “could be a major physiological advantage to growth under elevated CO<sub>2</sub> in this CAM bromeliad,” and this fact further suggests CAM species should “be considered in an agronomic context as potential sources of biomass production on arid, marginal lands.”

Sanchez-Guerrero (2009) grew cucumber plants from seed to maturity in standard perlite bags within climate-controlled greenhouses at Almeria, Spain, and the plants were “fertigated” (fertilized and irrigated) via a nutrient-solution drip system regulated to maintain the same electrical conductivity in the leached solution draining from the perlite bags of each greenhouse. One of the greenhouses was supplied with extra CO<sub>2</sub> during daylight hours (through outlets below each plant) when the greenhouse side vents were closed or when the roof vent was less than 20% of full opening, resulting in a mean daytime concentration of about 450 ppm around the plants, approximately 100 ppm more than the CO<sub>2</sub> concentration around the plants in the other greenhouse.

The total season-long yield of the CO<sub>2</sub>-enriched cucumber crop was increased by 19% by the extra

100 ppm of CO<sub>2</sub> supplied to it during daylight hours, and the overall water use efficiency of the CO<sub>2</sub>-enriched plants, based on the amount of water supplied to them, was about 40% higher. The five Spanish scientists conclude their study “confirms the potential interest of using moderate CO<sub>2</sub> enrichment strategies in greenhouses located in areas such as the Mediterranean basin, where the agricultural sector is facing scarce and declining water resources, and needs to drastically reduce the contamination due to fertilizer emission to ensure the sustainability of greenhouse production.”

Shimono *et al.* (2010) write, “by 2050, the world’s population will have increased by about 37%, from the current level of 6.7 billion to an estimated 9.2 billion (UN, 2009), with a corresponding increase in global food demand.” They also note “about 0.6 billion Mg of rice is produced annually from an area of 1.5 million km<sup>2</sup>, making rice one of the most important crops for supporting human life,” especially, as noted by Pritchard and Amthor (2005), since it supplies the planet’s human population with an estimated 20% of its energy needs (on a caloric basis) and 14% of its protein requirements (on a weight basis).

The six scientists further note “rice production depends heavily on water availability,” stating “irrigated lowlands account for 55% of the total area of harvested rice and typically produce two to three times the crop yield of rice grown under non-irrigated conditions (IRRI, 2002).” And because mankind’s demand for ever-greater quantities of water will continue to rise, due to the need to adequately feed the world’s growing numbers, they conclude, “efficient use of water will thus be essential for future rice production.”

To determine how the ongoing rise in the air’s CO<sub>2</sub> content may impact agriculture in this regard, the Japanese researchers conducted a two-year free-air CO<sub>2</sub> enrichment (FACE) study in fields at Shizukuishi, Iwate (Japan) to learn how elevated CO<sub>2</sub> may reduce crop water use via its impact on the leaf stomatal conductances (*gs*) of three varieties of rice (*Oryza sativa* L.): early maturing Kirara397, intermediate-maturing Akitakomachi, and latest-maturing Hitomebore.

In response to the 53% increase in daytime atmospheric CO<sub>2</sub> concentration employed in their experiments, Shimono *et al.* determined “the reduction in *gs* due to elevated CO<sub>2</sub> was similar across measurements, averaging around 20% in the morning, 24% around noon and 23% in the afternoon across all growth stages.” In addition, “there was no

significant CO<sub>2</sub> x cultivar interaction.” Therefore, with the concomitant increase in grain yield that results from atmospheric CO<sub>2</sub> enrichment, a continuation of the historical and still-ongoing rise in the air’s CO<sub>2</sub> content will play a major role in enabling the world to meet its food needs without having to lay claim to all of the planet’s remaining freshwater resources and much of its undeveloped land.

Expanding on this thesis, Fereres *et al.* (2011) write, “forecasts on population growth and economic development indicate that there will be substantial increases in food demand for the forthcoming decades,” and as a result, “food security has not only moved to the forefront of agricultural research, but is now perceived as an important topic for more fundamental research,” citing numerous items published in *Nature* (2010) and *Science* (2010). They add, “the question of whether there will be enough food in the future should immediately be followed by the question: Will there be enough water to produce sufficient food?” They state, “given the competition for water faced by the agricultural sector, and the uncertainties associated with climate change, improving the efficiency of water use in both rain-fed and irrigated systems is the main avenue to face the challenge.”

As to developing new sources of water, or transferring water from one place to another, in order to increase food production where water shortages exist, the three Spanish researchers state such measures have “limited potential in some areas” but are “no longer possible in other world regions.” They do note, however, many agriculturalists have significantly increased crop water use efficiency “by reducing water losses (and some of the water consumed in evaporation from soil) through improved agronomy and engineering of irrigation systems.” But they lament the fact, as they put it, “science has been much less successful so far in reducing the water consumed in transpiration.”

Fortunately, mankind has had a measurable amount of success in this area, albeit unintentionally and unknowingly. The extraction of fossil fuels from the crust of the Earth has provided coal, gas, and oil to fuel the engines of industry, and the carbon dioxide emitted to the air in the combustion process has raised the atmosphere’s CO<sub>2</sub> concentration by some 40% since the inception of the Industrial Revolution. That phenomenon has had two major effects on man’s production of food: It has increased significantly the leaf photosynthetic rates of crops, and it has reduced significantly their transpiration rates, which has led to

significant increases in leaf water use efficiency, the amount of biomass produced per unit of water transpired in the process.

In spite of these well-documented facts, to quote Morgan *et al.* (2011), “many believe that CO<sub>2</sub>-induced reductions in transpiration at the leaf level will be largely offset at the canopy level by increases in leaf area,” and “global warming is predicted to induce desiccation in many world regions through increases in evaporative demand.”

In a real-world test of these two potentially negative phenomena in a prairie heating and CO<sub>2</sub> enrichment (PHACE) experiment conducted in a native mixed-grass prairie in Wyoming (USA), Morgan *et al.* found the positive effects of elevated CO<sub>2</sub> prevailed, indicating, they write, “in a warmer, CO<sub>2</sub>-enriched world, both soil water content and productivity in semi-arid grasslands may be higher than previously expected,” providing what Baldocchi (2011) described as “one of the first and best views of how a mixed-grass ecosystem growing in a semi-arid climate will respond to future CO<sub>2</sub> and climatic conditions.”

A full decade earlier, Robock *et al.* (2000) had already developed a massive collection of soil moisture data from more than 600 stations spread across a variety of climatic regimes, including the former Soviet Union, China, Mongolia, India, and the United States. In analyzing those observations, they determined, “in contrast to predictions of summer desiccation with increasing temperatures, for the stations with the longest records, summer soil moisture in the top one meter has increased while temperatures have risen.”

Allen *et al.* (2011) write, “plants of the C<sub>4</sub> photosynthetic pathway have a CO<sub>2</sub>-concentrating mechanism that overcomes limitations of low atmospheric CO<sub>2</sub>” and thereby provides them with “a near-saturating photosynthetic capability at current atmospheric CO<sub>2</sub>.” In this circumstance, they note, “a rise in atmospheric CO<sub>2</sub> will theoretically have a limited direct impact on C<sub>4</sub> photosynthesis.” However, “a number of C<sub>4</sub> crop plants express a positive response to elevated growth CO<sub>2</sub>, although to a smaller extent compared to C<sub>3</sub> plants,” citing the analyses of Kimball (1993) and Poorter *et al.* (1996).

To test plants’ reaction to drought stress, the four researchers planted seeds of maize (*Zea mays* L. cv. Saturn Yellow) and grain sorghum (*Sorghum bicolor* L. cv. DeKalb 28E) in pots and grew them for 39 days in sunlit controlled-environment chambers at 360 and 720 ppm CO<sub>2</sub> concentrations. Throughout this period, canopy net photosynthesis and evapotranspiration

were measured and summarized daily from 0800 to 1700 hours. Irrigation was withheld from matched pairs of treatments starting 26 days after sowing, and biomass determinations were made at 34 and 39 days after sowing for maize and grain sorghum, respectively.

The four researchers found for both maize and grain sorghum, there was a “maintenance of relatively high canopy photosynthetic rates in the face of decreased transpiration rates [that] resulted in enhanced water use efficiency when these plants were grown at elevated CO<sub>2</sub> of 720 ppm, but not at 360 ppm.” Thus they demonstrated “both plants maintained growth better at double-ambient CO<sub>2</sub> than their counterparts at ambient CO<sub>2</sub> in the presence of drought,” such that reductions in total above-ground biomass due to drought were 42% for maize and 36% for sorghum at ambient CO<sub>2</sub>, but only 18% for maize and 14% for sorghum at double-ambient CO<sub>2</sub>. In discussing their findings, Allen *et al.* state they “agree with Leakey (2009) that drought stress in C<sub>4</sub> crop plants can be ameliorated at elevated CO<sub>2</sub> as a result of lower stomatal conductance and sustained intercellular CO<sub>2</sub>.”

As the CO<sub>2</sub> content of the air continues to rise, nearly all agricultural plants will respond favorably by exhibiting increases in water use efficiency. It is thus likely food and fiber production will increase on a worldwide basis, even in areas where productivity is severely restricted by limited availability of soil moisture. Global agricultural productivity should rise in tandem with future increases in the atmosphere’s CO<sub>2</sub> concentration.

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### 2.33.2 Grasslands

- As the air's CO<sub>2</sub> content continues to rise, nearly all of Earth's grassland species likely will experience increases in both productivity and water use efficiency, even if available moisture decreases. The CO<sub>2</sub>-induced increases in water use efficiency likely will allow grassland species to expand their ranges into desert areas where they previously could not survive due to lack of sufficient moisture, thereby contributing to a greater "greening of the globe."

As the air's CO<sub>2</sub> content rises, most plants exhibit increased rates of net photosynthesis and biomass production. Moreover, on a per-unit-leaf-area basis, they often lose less water via transpiration, as they tend to display lower stomatal conductances at elevated CO<sub>2</sub>. Hence, the amount of carbon gained per unit of water lost per unit leaf area—water use efficiency—should increase dramatically as the air's CO<sub>2</sub> content continues to rise. This section summarizes the results of recent studies of this phenomenon in grassland species.

Grunzweig and Korner (2001) grew model grasslands representative of the semi-arid Negev of Israel for five months at atmospheric CO<sub>2</sub> concentrations of 440 and 600 ppm. The grasslands exhibited cumulative water-use efficiencies 17 and 28% greater, respectively, than control communities grown at 280 ppm CO<sub>2</sub>. Similarly, Szente *et al.* (1998) report a doubling of the atmospheric CO<sub>2</sub> concentration increased the water use efficiency two C<sub>3</sub> grasses and two broad-leaved species common to the loess grasslands of Budapest by 72 and 266%, respectively. Leymarie *et al.* (1999) calculated twice-ambient CO<sub>2</sub> concentrations increased the water use efficiency of the herbaceous weedy species *Arabidopsis thaliana* by 41 and 120% under well-watered and water-stressed conditions, respectively. Other CO<sub>2</sub>-induced increases in C<sub>3</sub> plant water use efficiency were documented by Clark *et al.* (1999) for several New Zealand pasture species and by Roumet *et al.* (2000) for various Mediterranean herbs.

Elevated CO<sub>2</sub> also has been shown to increase the water use efficiency of C<sub>4</sub> grassland species. Adams *et al.* (2000), for example, report twice-ambient CO<sub>2</sub> concentrations enhanced the daily water use efficiency of a C<sub>4</sub> tallgrass prairie in Kansas (USA) dominated by *Andropogon gerardii*. LeCain and Morgan (1998) also documented enhanced water-use efficiencies for six C<sub>4</sub> grasses grown with twice-ambient CO<sub>2</sub> concentrations. Seneweera *et al.* (1998)

report a 650-ppm increase in the air's CO<sub>2</sub> content dramatically increased the water use efficiency of the perennial C<sub>4</sub> grass *Panicum coloratum*.

As the air's CO<sub>2</sub> content continues to rise, nearly all of Earth's grassland species—including both C<sub>3</sub> and C<sub>4</sub> plants—likely will experience increases in water use efficiency. The productivity of the world's grasslands also should increase, even if available moisture decreases in certain areas. Such CO<sub>2</sub>-induced increases in water use efficiency likely will allow grassland species to expand their ranges into desert areas where they previously could not survive due to lack of sufficient moisture, contributing to a greater "greening of the globe."

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### 2.33.3 Trees

- As the air's CO<sub>2</sub> content continues to rise, nearly all the planet's trees will exhibit increases in water use efficiency, enabling them to expand into areas where they previously could not grow due to insufficient available moisture. Earth will gradually acquire a much greener terrestrial cover with a significantly greater carbon sequestering capacity over time.

When the atmosphere's CO<sub>2</sub> concentration is increased, most plants exhibit increased rates of net photosynthesis and biomass production. In addition, they often lose less water via transpiration, as they tend to exhibit lower leaf stomatal conductances. Therefore, the amount of carbon they gain per unit of water lost per unit leaf area—water use efficiency—should increase significantly as the air's CO<sub>2</sub> content rises, as is described in this review of experimental results pertaining to this phenomenon in trees.

The effect of elevated atmospheric CO<sub>2</sub> concentrations on the water use efficiencies of trees is clearly positive, having been documented in numerous single-species studies of longleaf pine (Runion *et al.*, 1999), red oak (Anderson and Tomlinson, 1998), scrub oak (Lodge *et al.*, 2001), silver birch (Rey and Jarvis, 1998), beech (Bucher-Wallin *et al.*, 2000; Egli *et al.*, 1998), sweetgum (Gunderson *et al.*, 2002; Wullschlegel and Norby, 2001), and spruce (Robertz and Stockfors, 1998). In a multispecies study performed by Tjoelker *et al.* (1998), seedlings of quaking aspen, paper birch, tamarack, black spruce, and jack pine, grown at 580 ppm CO<sub>2</sub> for three months, displayed water use efficiencies 40 to 80% larger than those exhibited by their respective controls grown at 370 ppm CO<sub>2</sub>.

Similar results also have been obtained when trees were exposed to different environmental stresses. In a study conducted by Centritto *et al.* (1999), for example, cherry seedlings grown at twice-ambient levels of atmospheric CO<sub>2</sub> displayed water use efficiencies 50% greater than their ambient controls, regardless of soil moisture status. In the study of Wayne *et al.* (1998), yellow birch seedlings grown at 800 ppm CO<sub>2</sub> had water use efficiencies 52 and 94% greater than their respective controls when subjected to uncharacteristically low and high air temperature regimes, respectively.

In some parts of the world, perennial woody species have been exposed to elevated atmospheric CO<sub>2</sub> concentrations for decades, due to their proximity to CO<sub>2</sub>-emitting springs and vents in the

Earth, allowing scientists to assess the long-term effects of this phenomenon. In Venezuela, for example, the water use efficiency of a common tree exposed to a lifetime atmospheric CO<sub>2</sub> concentration of approximately 1,000 ppm rose twofold and 19-fold during the local wet and dry seasons, respectively (Fernandez *et al.*, 1998). Similarly, Bartak *et al.* (1999) report 30-year-old *Arbutus unedo* trees growing in central Italy at a lifetime aerial CO<sub>2</sub> concentration around 465 ppm exhibited water use efficiencies 100% greater than control trees growing at a lifetime CO<sub>2</sub> concentration of 355 ppm. Also, two species of oak in central Italy that had been growing for 15 to 25 years at an atmospheric CO<sub>2</sub> concentration ranging from 500 to 1,000 ppm displayed “such marked increases in water use efficiency under elevated CO<sub>2</sub>,” the scientists who studied them declare, this phenomenon “might be of great importance in Mediterranean environments in the perspective of global climate change.” The work of Blaschke *et al.* (2001) and Tognetti *et al.* (1998) suggest similar conclusions.

Some scientists also have looked to the past and determined the positive impact the historic rise in the air's CO<sub>2</sub> content already has had on plant water use efficiency. Duquesnay *et al.* (1998), for example, examined tree-ring data derived from beech trees and found the water use efficiency of such trees in north-eastern France increased by about 33% over the past century. Similarly, Feng (1999) used tree-ring chronologies from trees in western North America to calculate a 10 to 25% increase in tree water use efficiency from 1750 to 1970, during which time the atmospheric CO<sub>2</sub> concentration rose by approximately 16%.

Knapp *et al.* (2001) developed tree-ring chronologies from western juniper stands located in Oregon (USA) for the past century, determining growth recovery from drought was much greater in the latter third of their chronologies (1964–1998) than in the first third (1896–1930). The researchers suggest the greater atmospheric CO<sub>2</sub> concentrations of the latter period allowed the trees to recover more quickly from water stress. Beerling *et al.* (1998) grew *Ginkgo* saplings at 350 and 650 ppm CO<sub>2</sub> for three years and found elevated atmospheric CO<sub>2</sub> concentrations reduced leaf stomatal densities to values comparable to those measured on fossilized *Ginkgo* leaves dating back to the Triassic and Jurassic periods, implying greater water use efficiencies for those times too.

Tognetti *et al.* (2001) grew five-year-old seedlings of two olive cultivars in pots placed within free-air CO<sub>2</sub> enrichment (FACE) arrays maintained at

atmospheric CO<sub>2</sub> concentrations of 360 and 560 ppm for seven to eight months in a study designed to evaluate the effects of elevated CO<sub>2</sub> on gas exchange in this economically important tree species. The elevated CO<sub>2</sub> enhanced rates of net photosynthesis by an average of 38% in both cultivars while reducing stomatal conductances by an average of 30%. Instantaneous water use efficiency rose by approximately 80% in both cultivars, suggesting that as the air's CO<sub>2</sub> content continues to rise, olive trees growing in semi-arid Mediterranean-type climates should be able to cope with recurring drought conditions that are common in such areas.

Centritto *et al.* (2002) grew peach (*Prunus persica*) seedlings at atmospheric CO<sub>2</sub> concentrations of 350 and 700 ppm for one full year in two "growth tunnels," then transferred them to pots and placed them in open-top chambers having the same CO<sub>2</sub> concentrations for an additional three months, during the final four weeks of which half of the seedlings in each CO<sub>2</sub> treatment were allowed to "dry-down." This enabled the three researchers to investigate the interactive effects of elevated CO<sub>2</sub> and water stress on both photosynthesis and growth.

The elevated CO<sub>2</sub> stimulated net photosynthesis rates by about 60% in the well-watered seedlings. Under drought conditions, however, the relative photosynthetic stimulation increased to as much as 180%, ameliorating the negative effect of water stress. In addition, elevated CO<sub>2</sub> increased whole-plant water use efficiency by 51 and 63% in the well-watered and water-stressed seedlings, respectively. Thus, as the air's CO<sub>2</sub> content increases, peach seedlings likely will exhibit increased rates of both net photosynthesis and biomass production. In addition, they likely will be better able to deal with intermittent periods of water shortage without compromising overall productivity and growth. Peach production can be expected to increase as the atmospheric CO<sub>2</sub> concentration continues to rise.

Arneeth *et al.* (2002) describe how they developed 20 tree-ring <sup>13</sup>C/<sup>12</sup>C chronologies from *Pinus sylvestris* (Scots pine) trees at five locations along a 1,000-km north-south transect running through central Siberia they converted into plant isotopic discrimination ( $\delta^{13}\text{C}_c$ ) values. Based on these data, they conclude in 17 of the 20 samples the trees'  $\delta^{13}\text{C}_c$  had declined during the past 150 years, "particularly so during the second half of the twentieth century." Based on a model of stomatal behavior combined with a process-oriented photosynthesis model, they deduce "this trend indicates a long-term decrease in canopy stomatal conductance, probably in

response to increasing atmospheric CO<sub>2</sub> concentrations." They conclude their observations suggest "increased water use efficiency for Scots pine in central Siberia over the last century."

Centritto (2002) grew peach seedlings for two years in pots placed within open-top chambers of either ambient or CO<sub>2</sub>-enriched air (350 or 700 ppm, respectively) located inside a glasshouse, where they were continuously maintained at optimum soil fertility and, for the entire first growing season, at optimum soil water availability. In the second growing season, water was withheld from half the seedlings for four weeks.

At the end of the study, there were no CO<sub>2</sub>-induced differences in the basal diameters of the seedlings. In terms of total dry weight, however, the elevated CO<sub>2</sub> treatment enhanced the growth of the water-stressed seedlings by 30% and the growth of the well-watered seedlings by 35%, which was largely a consequence of increased height growth. In addition, Centritto found no evidence of any downward acclimation of photosynthesis in the seedlings grown at elevated CO<sub>2</sub>, nor was there any downward acclimation in rubisco carboxylation efficiency nor in the maximum RuBP regeneration capacity mediated by electron transport. There were also no significant effects of elevated CO<sub>2</sub> on stomatal conductance in either of the two water treatments. But the CO<sub>2</sub>-induced increase in plant growth brought a complementary increase in seedling water use efficiency, even though there was no difference in total water uptake between the two CO<sub>2</sub> treatments. In a world of the future where atmospheric CO<sub>2</sub> concentration is approximately doubled, young peach trees likely will produce about a third more growth on the same amount of water as they did at the turn of the century.

As part of the long-term (November 1987 to January 2005) sour orange tree study conducted at the U.S. Water Conservation Laboratory in Phoenix, Arizona (Idso and Kimball, 2001; Kimball *et al.*, 2007), Leavitt *et al.* (2003) report the results of a multifaceted investigation of a phenomenon never before assessed in this long-term experiment: the effects of a 75% increase in the air's CO<sub>2</sub> content on the efficiency with which well-watered and well-fertilized sour orange trees utilize water. It was based, as the six scientists noted, "on the conceptual framework developed by Farquhar *et al.* (1982), who defined intrinsic water use efficiency (iWUE) as the ratio of the photosynthetic uptake of CO<sub>2</sub> through leaf stomata to the simultaneous transpirational loss of water vapor through the same [stomatal] openings."

This ratio may be experimentally evaluated by measuring stable-carbon isotopes of various plant tissues and the air to which those tissues were exposed during their development. In this study, the plant materials utilized were leaves that had been collected every two months throughout 1992 and on three occasions in 1994–95, plus wood samples extracted five years later from north-south- and east-west-oriented wood cores that passed through the centers of each of the eight trees' trunks at a height of 45 cm above the ground. The average result of these measurements, evaluated within the context described by Farquhar *et al.*, was, as Leavitt *et al.* report, “an 80% increase in [water use efficiency] in response to the [75%] increase in atmospheric CO<sub>2</sub> concentration employed in the study.”

This result is interesting for a number of reasons. First, it suggests a doubling of the air's CO<sub>2</sub> content likely would bring more than a doubling of the trees' water use efficiency. Second, as the six scientists note, “this increase in sour orange tree iWUE is identical to the long-term CO<sub>2</sub>-induced increase in the trees' production of wood and fruit biomass,” as documented by Idso and Kimball (2001), suggesting a doubling of the air's CO<sub>2</sub> content should produce more than a doubling of the trees' total productivity, which further suggests the carbon sequestration potential of land planted to sour orange trees, and perhaps many other tree species, will increase dramatically in a CO<sub>2</sub>-enriched world.

Third, Leavitt *et al.* note the CO<sub>2</sub>-induced increase in sour orange tree water use efficiency is also identical “to the increase in the mean iWUE reported for 23 groups of naturally occurring trees scattered across western North America that was caused by the historical rise in the air's CO<sub>2</sub> content that occurred between 1800 and 1985,” as documented by Feng (1999), who further noted these iWUE trends in trees “are largely caused by the anthropogenic increase of the atmospheric CO<sub>2</sub> concentration,” concluding this phenomenon “would have caused natural trees in arid environments to grow more rapidly, acting as a carbon sink for anthropogenic CO<sub>2</sub>.” In addition, Leavitt *et al.* note “even greater water use efficiency responses have been observed in European tree-ring studies,” citing Bert *et al.* (1997) with white fir and Hemming (1998) with beech, oak, and pine trees. With respect to what these observations portend for the decades ahead, Leavitt *et al.* state, “the ongoing rise in the air's CO<sub>2</sub> content could continue to do the same for Earth's trees in the future,” dramatically increasing their productivity and the efficiency with which they utilize

water to achieve vastly enhanced growth rates.

As for how general this phenomenon could be expected to be, Saxe *et al.* (1998) determined in a comprehensive review of the scientific literature, “close to a doubling” of the air's CO<sub>2</sub> concentration leads to an approximate 50% increase in the biomass production of angiosperm trees and a 130% increase in the biomass production of coniferous species. With sour orange trees projected to experience just slightly more than a 100% increase in wood and fruit production in response to a doubling of the air's CO<sub>2</sub> concentration, the results of the Phoenix study fall well within the mid-range results typical of most other trees that have been similarly studied.

In light of these many empirical observations, one can confidently expect the growth rates of Earth's trees to increase dramatically as the air's CO<sub>2</sub> content continues to climb; this phenomenon, in turn, should enable them to sequester increasingly greater amounts of carbon. In addition, as the planet's trees become increasingly efficient at utilizing water, one could expect to see them rapidly expand into areas that are currently too dry to support their growth and reproduction, and this phenomenon also should increase the magnitude of carbon sequestration by Earth's trees. Hence, as time progresses, the planet's trees, if not destroyed by mankind's cutting and burning them, should provide an ever-increasing brake upon the rate of rise of the air's CO<sub>2</sub> content.

Waterhouse *et al.* (2004) determined the intrinsic water use efficiency (iWUE) responses of three tree species growing across northern Europe—pedunculate oak (*Quercus robur* L.), common beech (*Fagus sylvatica* L.), and Scots pine (*Pinus sylvestris* L.)—to the increase in the air's CO<sub>2</sub> concentration experienced between 1895 and 1994, using parameters derived from measurements of stable carbon isotope ratios of trunk cellulose. They report, “all species at all the sites show a long-term increase in their values of iWUE during the past century,” and “the main cause of this common behavior is likely to be the increase in atmospheric CO<sub>2</sub> concentration.”

Linearly extrapolating these responses, which occurred over a period of time when the air's CO<sub>2</sub> concentration rose by approximately 65 ppm, to what would be expected for the more common 300-ppm increase employed in the majority of atmospheric CO<sub>2</sub> enrichment experiments, the iWUE increases they derived amount to +158 ± 14% for the oak trees (mean ± standard error for the five sites studied), +195% for the pine trees, and +220% for the beech trees, as best as can be determined from the graphs of their results. These responses are huge and probably

not due to rising CO<sub>2</sub> alone, but to the positive synergism that occurs when the air's CO<sub>2</sub> content and temperature rise together, as these parameters have done over the past century or so.

Peterson and Neofotis (2004) grew velvet mesquite (*Prosopis velutina* Woot.) seedlings for six weeks from their time of planting (as seeds) in small pots within environmentally controlled growth chambers maintained at atmospheric CO<sub>2</sub> concentrations of 380 and 760 ppm and two levels of water availability (high and low). Although they did not see a significant CO<sub>2</sub>-induced increase in plant growth, they report by the end of their six-week study there was a highly significant reduction of approximately 41% in the volume of water transpired by *P. velutina* in response to the experimental doubling of the air's CO<sub>2</sub> content. "This large reduction in whole-plant water use," they write, "occurred because the reduction in transpiration per unit leaf area at elevated CO<sub>2</sub> was not offset by a proportional increase in total leaf area."

The two scientists from the Biosphere 2 Center near Oracle, Arizona (USA) state their findings suggest "under a future [high-CO<sub>2</sub>] scenario, seedlings may deplete soil moisture at a slower rate than they do currently," and "this could facilitate seedling survival between intermittent rain events," noting their work "corroborates the conclusions of Polley *et al.* (1994, 1999, 2003) that increasing levels of atmospheric CO<sub>2</sub> may facilitate the establishment of mesquite seedlings through a reduction in soil water depletion." Peterson and Neofotis note, "mesquites and other woody species in the semiarid southwestern United States have shown substantial increases in population density and geographic range since Anglo-American settlement of the region approximately 120 years ago," citing Van Auken and Bush (1990), Gibbens *et al.* (1992), Bahre and Shelton (1993), Archer (1995), Boutton *et al.* (1999), Van Auken (2000), Ansley *et al.* (2001), Wilson *et al.* (2001), and Biggs *et al.* (2002).

Saurer *et al.* (2004), measured carbon isotope ratios in the rings of coniferous trees from northern Eurasia—including the three genera *Larix*, *Picea* and *Pinus*—across a longitudinal transect covering the entire super-continent in the latitude range from 59 to 71°N, to determine the change in intrinsic water use efficiency (Wi, the amount of water loss at the needle level per unit of carbon gain) experienced by the trees between the two 30-year periods 1861–1890 and 1961–1990.

They found the "increasing CO<sub>2</sub> in the atmosphere resulted in improved intrinsic water use

efficiency," such that "125 out of 126 trees showed increasing Wi from 1861–1890 to 1961–1990, with an average improvement of 19.2 ± 0.9%." The three Swiss scientists state their results suggest the trees they studied "are able to produce the same biomass today [as they did 100 years ago] but with lower costs in terms of transpiration." This finding is highly significant, because some data had indicated recent warming in other longitudinal segments of the same latitude belt "may be accompanied by increased drought stress (Lloyd and Fastie, 2002)." The historical increase in the air's CO<sub>2</sub> content may have been helping those trees better cope with the newly established drought conditions.

Syvertsen and Levy (2005) reviewed what was known about salinity stress in citrus trees and how it may be modified by atmospheric CO<sub>2</sub> enrichment. They note, for example, rapidly growing plants almost always use more water than slower growing plants, and "in citrus, many vigorous rootstocks that produce fast-growing trees also tend to have poor salt tolerance (Castle *et al.*, 1993)," possibly because they accumulate more salt in their tissues because of their greater uptake of water. When growing plants in CO<sub>2</sub>-enriched air, however, plant stomatal conductance and water use often are decreased at the same time net photosynthesis and growth are increased, so, "elevated CO<sub>2</sub> almost always leads to higher water use efficiency as it disconnects rapid tree growth from high water use." Consequently, the two scientists explain, "if salt uptake is coupled with water uptake, then leaves grown at elevated CO<sub>2</sub> should have lower salt concentrations than leaves grown at ambient CO<sub>2</sub> (Ball and Munns, 1992)."

"As expected," Syvertsen and Levy write, "all citrus rootstock species studied increased growth and water use efficiency in response to elevated CO<sub>2</sub> that was twice ambient," and generally, but not always, "the salinity-induced accumulation of sodium (Na<sup>+</sup>) in leaves was less when seedlings were grown at elevated CO<sub>2</sub> than at ambient CO<sub>2</sub>." One exception, where Na<sup>+</sup> accumulation was not affected by elevated CO<sub>2</sub>, was Rangpur lime (*Citrus reticulata*); the scientists note this citrus variety is relatively salt-tolerant and another variety of the same species (Cleopatra mandarin) had lower leaf chloride (Cl<sup>-</sup>) concentrations in CO<sub>2</sub>-enriched air than in ambient air.

All the citrus trees tested to that point in time had exhibited increased growth rates and water use efficiencies when growing in CO<sub>2</sub>-enriched air. In addition, they generally experienced less salinity stress than when grown in lower-CO<sub>2</sub> ambient air. As

a result, the ongoing rise in the atmosphere's CO<sub>2</sub> concentration bodes well for the future vitality and productivity of the many varieties of citrus trees, which in turn bodes well for humanity.

Hietz *et al.* (2005) collected samples of wood from 37 tropical cedar (*Cedrela odorata* L.) trees that were between 11 and 151 years old in 2001 and from 16 big-leaf mahogany (*Swietenia macrophylla* King) trees that were between 48 and 126 years old at that time, from a rain forest in Aripuana, Brazil. They measured the wood samples' cellulose  $\delta^{13}\text{C}$  in 10-year growth increments. They found the cellulose  $\delta^{13}\text{C}$  decreased by 1.3 per mil in *Cedrela* and by 1.1 per mill in *Swietenia* over the past century, with the largest changes occurring during the last 50 years. Based on these data and known trends in atmospheric CO<sub>2</sub> and  $\delta^{13}\text{CO}_2$ , they calculated the intrinsic water use efficiency of the trees increased by 34% in *Cedrela* and by 52% in *Swietenia* over this period, which they state is about the same as what had been deduced from similar measurements of the wood of temperate trees (Freyer, 1979; Bert *et al.*, 1997; Feng, 1999).

The three researchers note, since "water is probably not a strong limiting factor in tropical rain forest trees," the increase in water use efficiency they discovered likely "translates mostly to increased carbon assimilation, which may explain the observed increase in tree growth and turnover (Phillips, 1996; Laurance *et al.*, 2004)" in such forests.

Defining intrinsic water use efficiency (iWUE) as the ratio of the photosynthetic uptake of CO<sub>2</sub> through leaf stomata to the simultaneous transpirational loss of water vapor through the stomata, Liu *et al.* (2007) evaluated this parameter based on  $\delta^{13}\text{C}$  measurements of cellulose extracted from the wood of tree-ring cores taken from living Qilian juniper (*Sabina przewalskii* Kom.) and Qinghai spruce (*Picea crassifolia* Kom.) trees, focusing on the period AD 1850–2000 at time resolutions of three years for juniper from the semi-arid Qilian Mountains, two years for juniper from the arid Qaidam Basin, and one year for spruce from both of the northwest China sites.

Overall, and based on means for the first and last decades of the study period, the seven Chinese researchers found "the iWUE values of the two species both showed long-term increases, by 33.6 and 37.4% for spruce in the arid and semi-arid areas, respectively, and by increases of 24.7 and 22.5% for juniper," noting "the main cause of this behavior is likely to be an increase in atmospheric CO<sub>2</sub> concentration," which for the start and end decades of

the study period rose from approximately 285 ppm to 362 ppm, about 27%.

Increases in the water use efficiencies of trees in arid and semi-arid regions must be considered a significant benefit. And in the case of the two species studied by Liu *et al.*, they note Qinghai spruce, in particular, "plays an important role in preventing soil erosion, regulating climate, and retaining ecological stability," citing Zhou and Li (1990) in this regard. This phenomenon is undoubtedly one of the chief reasons for the concomitant "greening of the Earth" that has been so evident in many historical studies of China and other parts of Asia.

Leal *et al.* (2008) obtained cores from eight to 20 black pine (*Pinus nigra*) trees growing at each of 28 sites within the Vienna basin of Austria in the European Eastern Alps during the summers of 1996 and 1997, focusing on trees possessing umbrella-like crowns (indicating water-limited conditions) growing on shallow and poor soils, in order to maximize their ring-width response to moisture availability. They discovered "a very clear change in the sensitivity of the growth rate of tree stems to water availability in the late 20th century," noting "trees previously sensitive to spring-summer drought show a lack of response to this climatic parameter in recent decades." Hence, they write, "tree-ring indices were larger in the second half of the 20th century than predicted given prevailing spring-summer drought conditions and the previous sensitivity of growth to these conditions." In addition, they found "a decrease in correspondence between the occurrence of extreme events in precipitation and rate of change of growth," such that "in the second half of the century this correspondence was not significant," and "recent extreme droughts did not result in the formation of very narrow rings, which means the droughts were not as limiting to tree growth as they had been in the past."

The five researchers suggest the greater atmospheric CO<sub>2</sub> concentrations of the latter decades of the twentieth century "induced improved water use efficiency enabling *P. nigra* growing in the Vienna basin to avoid the impact of recurrent dry conditions." This phenomenon also has been observed in many other parts of the world in a number of tree species.

Describing *Araucaria angustifolia* as "an indigenous conifer tree restricted to the southern region of South America that plays a key role in the dynamics of regional ecosystems where forest expansion over grasslands has been observed," Silva *et al.* (2009) studied various types of tree-ring data obtained from *A. angustifolia* trees growing in both

forest and grassland sites of southern Brazil. They compared changes in iWUE with historical changes in temperature, precipitation, and atmospheric CO<sub>2</sub> concentration over the past century.

During the past several decades, they write, “iWUE increased over 30% in both habitats [forests and grasslands],” and “this increase was highly correlated with increasing levels of CO<sub>2</sub> in the atmosphere.” However, tree growth remained rather stable over this latter period, due to lower-than-normal precipitation and higher-than-normal temperatures, which would normally tend to depress the growth of this species, as Katinas and Crisci (2008) described *A. angustifolia* as being “intolerant of dry seasons and requiring cool temperatures.” Therefore, Silva *et al.* conclude, “climatic fluctuations during the past few decades,” which normally would be expected to have been deleterious to the growth of *A. angustifolia*, seem to have had their growth-retarding effects “compensated by increases in atmospheric CO<sub>2</sub> and changes [i.e., increases] in iWUE.”

Wyckoff and Bowers (2010) note, “with continued increases in global greenhouse gas emissions, climate models predict that, by the end of the 21st century, Minnesota [USA] summer temperature will increase by 4–9°C and summer precipitation will slightly decrease,” citing Kling *et al.* (2003) and Christensen *et al.* (2007). They further note certain “forest models and extrapolations from the paleoecological record suggest that, in response to increased temperature and/or drought, forests may retreat to the extreme north-eastern parts of the state,” citing Pastor and Post (1998), Hamilton and Johnson (2002), and Galatowitsch *et al.* (2009).

Working with bur oak (*Quercus macrocarpa*) trees, Wyckoff and Bowers explored the likelihood of the latter of these two projections coming to pass by “(i) using tree rings to establish the relationship between drought and *Q. macrocarpa* growth for three sites along Minnesota’s prairie-forest border, (ii) calculating the current relationship between growth and mortality for adult *Q. macrocarpa* and (iii) using the distributions of current growth rates for *Q. macrocarpa* to predict the susceptibility of current populations to droughts of varying strength.” They also looked for “temporal trends in the correlation between *Q. macrocarpa* growth and climate, hypothesizing increases in CO<sub>2</sub> may lead to weaker relationships between drought and tree growth over time,” because atmospheric CO<sub>2</sub> enrichment typically leads to increases in plant water use efficiency, which generally makes them less susceptible to the deleterious impact of drought on growth.

The two University of Minnesota researchers discovered “the sensitivity of annual growth rates to drought has steadily declined over time as evidenced by increasing growth residuals and higher growth rates for a given PDSI [Palmer Drought Severity Index] value after 1950 [when the atmosphere’s CO<sub>2</sub> concentration rose by 57 ppm from 1950 to 2000] compared with the first half of the century [when the CO<sub>2</sub> increase was only 10 ppm].” Thus, they conclude, “for *Q. macrocarpa*, declining sensitivity of growth to drought translates into lower predicted mortality rates at all sites,” and “at one site, declining moisture sensitivity yields a 49% lower predicted mortality from a severe drought (PDSI = -8, on a par with the worst 1930s ‘American Dust Bowl’ droughts).” Hence, “the decreasing drought sensitivity of established trees may act as a buffer and delay the movement of the prairie-forest ecotone for many decades even in the face of climate change.”

Brienen *et al.* (2011) note water use efficiency is the ratio of photosynthesis (A) to transpiration (E), or the amount of carbon gained per unit of water used in the process of acquiring the carbon. They also define A/gs—where gs is stomatal conductance—to be intrinsic water use efficiency (Wi), and state, “an increase in Wi in response to increasing CO<sub>2</sub> since the industrial revolution has been found in nearly all temperate trees that have been studied,” citing Feng (1999), Saurer *et al.* (2004), and Nock *et al.* (2010). They decided to see if this is true also for tropical trees.

Noting “increases in Wi have been observed in short-term experiments of tree responses to elevated CO<sub>2</sub> (Norby *et al.*, 1999), and over long-time periods using records of δ<sup>13</sup>C in tree rings that reflect the global increase in atmospheric CO<sub>2</sub> (Feng, 1999; Waterhouse *et al.*, 2004),” Brienen *et al.* “analyzed carbon isotope ratios over the last 40 years in tree rings of *Mimosa acantholoba*, a tropical dry forest pioneer species,” in a study conducted “on the Pacific slope of the isthmus of Tehuantepec, close to the village of Nizanda in the state of Oaxaca, South Mexico (16°39’N, 95°00’W).” The three researchers, representing Austria, Mexico, and the United Kingdom, report the dry-forest tropical *M. acantholoba* trees “responded strongly to the increase in atmospheric CO<sub>2</sub> over the last four decades,” as their “Wi increased dramatically by 40%.”

Chen *et al.* (2011) write “Idso (1998) suggested that elevated CO<sub>2</sub> affects plant growth dependent upon plant water status: it has less effect on plants in the well-watered optimal growth phase, but exerts more effect under non-lethal dry conditions, and is

most beneficial to plants under severe drought conditions.” In a further assessment of this phenomenon, Chen *et al.* measured leaf transpiration rate ( $E$ ) and net photosynthetic rate ( $PN$ ) in *Populus euphratica* trees growing just within the northern edge of the Taklimakan Desert in Xinjiang, northwestern China, where the riparian trees dominate the indigenous vegetation because of their tolerance of severe drought and the high salinity and alkalinity of the region’s soils. They did so in four locations, where mean soil water contents at groundwater depths of 4.12, 4.74, 5.54, and 7.74 meters were 10.9, 9.5, 3.5, and 1.3%, respectively, making their measurements at atmospheric  $CO_2$  concentrations of either 360 or 720 ppm. They then calculated the trees’ water use efficiencies ( $WUE = PN/E$ ) when measured under the two atmospheric  $CO_2$  concentrations.

In the case of each  $CO_2$  concentration, the researchers found no statistical difference between the leaf water use efficiencies of the first three groundwater depths; but the mean WUE at the higher of the two  $CO_2$  concentrations was 44% greater than the mean measured at the lower  $CO_2$  concentration. The WUE of the lowest and driest of the four groundwater depths was statistically different from the WUEs of the other three groundwater depths, and the mean WUE of the trees growing under this most stressful condition when measured at the higher of the two  $CO_2$  concentrations was 86% greater than the mean measured at the lower  $CO_2$  concentration. Chen *et al.* conclude, with respect to the plant water use efficiency of *Populus euphratica* trees, those growing “under a mild water stress show a weak responsiveness, and those under a moderate drought stress display a strong responsiveness to  $CO_2$  enrichment.”

Soule and Knapp (2011) note, “in 2008, atmospheric  $CO_2$  concentrations from the Mauna Loa, Hawaii, Observatory records exceeded 385 ppm, representing a 22% increase since 1959,” and “as  $CO_2$  has increased, most tree species have been able to use water more efficiently,” as their “leaf stomatal apertures narrow during photosynthesis,” resulting in “less transpirational water loss per biomass gained.” The two researchers studied changes in and relationships among radial growth rates and the iWUE of ponderosa pine (*Pinus ponderosa*) trees, climate, and atmospheric  $CO_2$  concentration in the western United States since the mid-nineteenth century, developing tree-ring chronologies for eight sites in three climate regions and using carbon isotope data to calculate pentadal values of iWUE. They examined relationships among radial growth, climate,

iWUE, and  $CO_2$  via correlation and regression analyses.

Soule and Knapp report finding significant upward trends in iWUE at all sites, and they state, “despite an absence of climate changes that would favor growth,” upward radial growth trends occurred at five sites. In addition, they discovered the highest iWUE values “were recorded in the last pentad at six of eight sites and follow a positive quadratic progression at all sites, suggesting that future increases in iWUE are likely for ponderosa pine within our study regions as  $CO_2$  levels increase.” They found “significant improvements in radial growth rates during drought years after 1950,” when the air’s  $CO_2$  content rose at an accelerating rate.

The two U.S. researchers suggest “increased iWUE associated with rising  $CO_2$  can positively impact tree growth rates in the western United States and are thus an evolving component of forest ecosystem processes.” They conclude, “if potential climate changes lead to increasing aridity in the western United States, additional increases in iWUE associated with future increases in  $CO_2$  might ameliorate growth declines associated with drought conditions.”

Wang *et al.* (2012) introduce their treatment of the subject by noting iWUE “represents the ratio of photosynthetic assimilation ( $A$ ) to stomatal conductance ( $g_w$ ),” and “higher iWUE can result from reducing  $g_w$ , increasing  $A$ , or a combination of the two responses.” They also state, “empirical evidence from lab studies with a controlled  $CO_2$  concentration and from free-air  $CO_2$  enrichment (FACE) experiments have revealed significantly increased iWUE in response to rising  $CO_2$ ,” as demonstrated by the studies of Luo *et al.* (1996), Ainsworth and Rogers (2007), and Niu *et al.* (2011). They also note “tree-ring stable carbon isotope ratios ( $\delta^{13}C$ ) have proven to be an effective tool for evaluating variations in iWUE around the world,” citing Farquhar *et al.* (1989), Saurer *et al.* (2004), Liu *et al.* (2007), and Andreu *et al.* (2011). In addition, they report “during the past 100–200 years, most of the sampled forests demonstrated a trend of increasing iWUE, which paralleled the increasing atmospheric  $CO_2$ ,” citing Peñuelas *et al.* (2011) and references therein.

In November 2009, Wang *et al.* extracted two cores from the trunks of each of 17 dominant living Qinghai spruce (*Picea crassifolia*) trees at a site in the Xinglong Mountains in the eastern part of northwestern China (35°40’N, 104°02’E), obtaining from those cores precise ring-width measurements

they used to calculate yearly mean basal area growth increments. They used subsamples of the cores to conduct the stable carbon isotope analyses needed to obtain the  $\delta^{13}\text{C}$  data required to calculate iWUE over the course of their study period: 1800–2009. By calibrating the  $\delta^{13}\text{C}$  data against climatic data obtained at the nearest weather station over the period 1954–2009, they were able to extend the histories of major meteorological parameters back to 1800. And by comparing these weather data with the tree growth and water use efficiency data, they were able to interpret the impacts of climate change and atmospheric  $\text{CO}_2$  enrichment on spruce tree growth and water use efficiency.

For the arid region of northwestern China in which the spruce trees they studied were growing, Wang *et al.* found iWUE increased by approximately 40% between 1800 and 2009, rising very slowly for the first 150 years but then more rapidly to about 1975, and then faster still until 1998, when it leveled off for the remaining 11 years of the record. Commenting on the main cause of the increasing trend in iWUE from 1800 to 1998, they state it “is likely to be the increase in atmospheric  $\text{CO}_2$ ,” because “regression analysis suggested that increasing atmospheric  $\text{CO}_2$  explained 83.0% of the variation in iWUE from 1800 to 1998 ( $p < 0.001$ ).” Thereafter, they note, a substantial drought at the end of the record is probably what caused the leveling off of iWUE, which was also strong enough to cause a decline in yearly basal area growth increment, much like what occurred between 1923 and 1934, which they describe as “the most severe drought since 1800,” citing Fang *et al.* (2009).

Wang *et al.*'s study suggests the historical increase in the air's  $\text{CO}_2$  content over the course of the Industrial Revolution gradually but greatly enhanced the intrinsic water use efficiency of Qinghai spruce trees in northwest China, as well as their growth rates. During times of very severe drought stress, even this added help can fall short of what is needed to keep the trees from maintaining an exemplary rate of growth. Rising atmospheric  $\text{CO}_2$  concentrations see them through the times of severe stress to where they can once again grow exceedingly well once the drought is past.

Battipaglia *et al.* (2013) combined tree ring analyses with carbon and oxygen isotope measurements made at three free-air  $\text{CO}_2$  enrichment (FACE) sites—POP-EUROFACE in Italy, Duke FACE in North Carolina (USA), and ORNL in Tennessee (USA)—“to cover the entire life of the trees,” which they accomplished by using  $\delta^{13}\text{C}$  to assess carbon

isotope discrimination and changes in water use efficiency, while direct  $\text{CO}_2$  effects on stomatal conductance were explored using  $\delta^{18}\text{O}$  as a proxy.

The seven scientists report, “across all the sites, elevated  $\text{CO}_2$  increased  $^{13}\text{C}$ -derived water use efficiency on average by 73% for *Liquidambar styraciflua* [POP-EUROFACE, +200 ppm  $\text{CO}_2$ ], 77% for *Pinus taeda* [Duke FACE, +200 ppm  $\text{CO}_2$ ] and 75% for *Populus sp.* [ORNL, +153 ppm  $\text{CO}_2$ ], but through different ecophysiological mechanisms.” They state their findings provide “a robust means of predicting water use efficiency responses from a variety of tree species exposed to variable environmental conditions over time, and species-specific relationships that can help modeling elevated  $\text{CO}_2$  and climate impacts on forest productivity, carbon and water balances.”

As the  $\text{CO}_2$  content of the air continues to rise, nearly all Earth's trees will respond favorably through increases in water use efficiency. Woody species are likely to expand into areas where they previously could not exist due to limiting amounts of available moisture. One can expect Earth to become a greener biospheric body with greater carbon-sequestering capacity as the air's  $\text{CO}_2$  concentration continues to rise.

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### 2.34 Wood Density

- Atmospheric CO<sub>2</sub> enrichment tends to increase wood density in both seedlings and mature trees, increasing a number of strength properties of their branches and trunks. Different species of trees may respond somewhat differently to atmospheric CO<sub>2</sub> enrichment, and they can respond with still greater variety under different environmental conditions.

Numerous experiments have demonstrated trees grown in air enriched with CO<sub>2</sub> nearly always sequester more biomass in their trunks and branches than do trees grown in ambient air. Several studies also have looked at the effects of elevated CO<sub>2</sub> on the density of that sequestered biomass, some of which are summarized here.

Rogers *et al.* (1983) observed no difference in the wood density of loblolly pine (*Pinus taeda*) trees grown at 340 and 718 ppm CO<sub>2</sub> for 10 weeks; but they found a 33% CO<sub>2</sub>-induced increase in the wood density of sweetgum (*Liquidambar styraciflua*) trees grown at these concentrations for only eight weeks. Doyle (1987) and Telewski and Strain (1987) studied the same two tree species over three growing seasons in air of 350 and 650 ppm CO<sub>2</sub>, finding no effect of atmospheric CO<sub>2</sub> enrichment on the stem density of sweetgum, but a mean increase of 9% in the stem density of loblolly pine.

Conroy *et al.* (1990) grew seedlings of two *Pinus radiata* families at 340 and 660 ppm CO<sub>2</sub> for 114 weeks, finding CO<sub>2</sub>-induced trunk density increases for the two families of 5.4 and 5.6% when soil phosphorus was less than adequate and increases of 5.6 and 1.2% when it was nonlimiting. In a similar study, Hattenschwiler *et al.* (1996) grew six genotypes of clonally propagated four-year-old Norway spruce (*Picea abies*) for three years at CO<sub>2</sub> concentrations of 280, 420, and 560 ppm at three rates of wet nitrogen deposition. On average, they found wood density was 12% greater in the trees grown at

the two higher CO<sub>2</sub> concentrations than in the trees grown at 280 ppm.

Norby *et al.* (1996) grew yellow poplar or “tulip” trees (*Liriodendron tulipifera*) at ambient and ambient plus 300 ppm CO<sub>2</sub> for three years, during which time the wood density of the trees increased by approximately 7%. Tognetti *et al.* (1998) studied two species of oak tree—one deciduous (*Quercus pubescens*) and one evergreen (*Quercus ilex*)—growing in the vicinity of CO<sub>2</sub> springs in central Italy that raised the CO<sub>2</sub> concentration of the surrounding air by approximately 385 ppm. This increase in the air’s CO<sub>2</sub> content increased the wood density of the deciduous oaks by 4.2% and that of the evergreen oaks by 6.4%.

Telewski *et al.* (1999) grew loblolly pine trees for four years at ambient and ambient plus 300 ppm CO<sub>2</sub>. Wood density determined directly from mass and volume measurements was increased by 15% by the extra CO<sub>2</sub>, and average ring density determined by X-ray densitometry was increased by 4.5%.

Beismann *et al.* (2002) grew different genotypes of spruce and beech (*Fagus sylvatica*) seedlings for four years in open-top chambers maintained at atmospheric CO<sub>2</sub> concentrations of 370 and 590 ppm in combination with low and high levels of wet nitrogen application on both rich calcareous and poor acidic soils, to study the effects of these factors on seedling toughness (fracture characteristics) and rigidity (bending characteristics such as modulus of elasticity). They found some genotypes of each species were sensitive to elevated CO<sub>2</sub> while others were not. Similarly, some were responsive to elevated nitrogen deposition, but others were not. Moreover, such responses often were dependent upon soil type. Averaged across all tested genotypes, atmospheric CO<sub>2</sub> enrichment increased wood toughness in spruce seedlings grown on acidic soils by 12 and 18% at low and high levels of nitrogen deposition, respectively. In addition, atmospheric CO<sub>2</sub> enrichment increased this same wood property in spruce seedlings grown on calcareous soils by about 17 and 14% with low and high levels of nitrogen deposition, respectively. In contrast, elevated CO<sub>2</sub> had no significant effects on the mechanical wood properties of beech seedlings, regardless of soil type.

Kilpelainen *et al.* (2003) erected 16 open-top chambers within a 15-year-old stand of Scots pines growing on a nutrient-poor sandy soil of low nitrogen content near the Mekrijarvi Research Station of the University of Joensuu, Finland. Over the next three years they maintained the trees within these chambers in a well-watered condition, while they enriched the

air in half of the chambers to a mean daytime CO<sub>2</sub> concentration of approximately 580 ppm and maintained the air in half of each of the two CO<sub>2</sub> treatments at 2°C above ambient. In the ambient temperature treatment the 60% increase in the air's CO<sub>2</sub> concentration significantly increased latewood density by 27% and maximum wood density by 11%. In the elevated-temperature treatment the 60% increase in the air's CO<sub>2</sub> concentration significantly increased latewood density by 25% and maximum wood density by 15%. These changes led to mean overall CO<sub>2</sub>-induced wood density increases of 2.8% in the ambient-temperature treatment and 5.6% in the elevated-temperature treatment.

Kostiainen *et al.* (2004) investigated the effects of elevated CO<sub>2</sub> (doubled concentration: 720 ppm vs. 360 ppm) and elevated nutrient input to soil (described as “heavy fertilization,” i.e., “higher than used in forestry in practice”) on a number of wood properties of 40-year-old Norway spruce (*Picea abies* L. Karst.) trees enclosed by open-top chambers for three years. They report previous data from this long-term study “showed fertilization decreased wood density (Makinen *et al.*, 2002),” and in the presence of elevated CO<sub>2</sub>, such was still found to be the case in the new study, but only for earlywood density (a mean decrease of 3.8% over the three years of the study). In the case of latewood density, the extra CO<sub>2</sub> supplied to the trees overrode the negative effect of heavy fertilization and increased mean wood density by 4.6%. Moreover, in the treatment where no extra nutrients were supplied to the trees, both earlywood and latewood density were increased by the doubling of the air's CO<sub>2</sub> concentration: by 4.8% in the case of earlywood density and by 2.0% in the case of latewood density. Thus, under normal growing conditions, a doubling of the air's CO<sub>2</sub> concentration likely would increase the wood density of Norway spruce trees by about 2–5%.

Buitenwerf *et al.* (2012) analyzed changes in woody-plant density at three sites in South African savannas where the normal disturbance regime (fire and herbivores) was kept constant for either 30 or 50 years, noting “if global drivers had significant effects on woody plants, we would expect significant increases in tree densities and biomass over time under the constant disturbance regime.”

The four South African scientists report for the more arid savannas they analyzed there was no indication of global drivers promoting an increase in wood density over the period of their study. However, they found wood density tripled in a mesic savanna between the 1970s and 1990s; in another mesic

savanna it doubled from the mid-1990s to 2010; and “aerial photograph analysis on adjacent non-cleared areas showed an accompanying 48% increase in woody cover.” Buitenwerf *et al.* say their analysis “has shown significant increase in tree densities and stature that are consistent with global drivers promoting woody thickening.” They conclude “the only plausible candidate in the experimental areas is increasing CO<sub>2</sub> since there were no significant temperature or rainfall trends over the last 50 years.”

These observations make it clear different species of trees sometimes respond differently to atmospheric CO<sub>2</sub> enrichment, and they respond with still greater variety under different sets of environmental conditions. In general, however, atmospheric CO<sub>2</sub> enrichment tends to increase wood density in both seedlings and mature trees, thereby also increasing strength properties of their branches and trunks.

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