The Influence of CO_2 on Plant Antioxidants

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THE INFLUENCE OF CO_2 ON PLANT ANTIOXIDANTS

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Environmental stresses induced by exposure to pollutants, drought, intense solar radiation and high air or water temperatures generate highly-reactive oxygenated compounds that damage both terrestrial and aquatic plants. Ameliorating these stresses typically involves the production of antioxidant enzymes that scavenge and detoxify the highly-reactive oxygenated compounds. Hence, when stresses are present, concentrations and/or activities of antioxidants in plants are generally observed to be high; and a good question to ask, therefore, is how atmospheric CO_2 enrichment impacts this relationship and what the observed results imply. A number of researchers have done just that, and in this summary we highlight what they have learned.

In a study of two soybean genotypes, Pritchard *et al.* (2000)[1] reported that three months' exposure to twice-ambient CO_2 concentrations reduced the activities of superoxide dismutase and catalase by an average of 23 and 39%, respectively. Likewise, Polle *et al.* (1997)[2] showed that two years of atmospheric CO_2 enrichment reduced the activities of several key antioxidative enzymes, including catalase and superoxide dismutase, in beech seedlings. And Schwanz and Polle (1998)[3] demonstrated that this phenomenon can persist *indefinitely*, for they discovered similar reductions in these same enzymes in mature oak trees that had been growing near natural CO_2-emitting springs for 30 to 50 years.

> "CO_2-advantaged" plants will be able to funnel more of their limited resources into the production of other plant tissues or processes essential to their continued growth and development.

The standard interpretation of these results is that the observed reductions in the activities of antioxidative enzymes under CO_2-enriched conditions imply that plants exposed to higher-than-current atmospheric CO_2 concentrations experience less oxidative stress and thus have a reduced need for antioxidant protection. This conclusion further suggests that "CO_2-advantaged" plants will be able to funnel more of their

[1] http://www.co2science.org/articles/V3/N37/B1.php
[2] http://www.co2science.org/articles/V1/N7/B5.php
[3] http://www.co2science.org/articles/V2/N10/B5.php

limited resources into the production of other plant tissues or processes essential to their continued growth and development.

On the other hand, when oxidative stresses do occur under high CO2 conditions, the enhanced rates of photosynthesis and carbohydrate production resulting from atmospheric CO_2 enrichment generally enable plants to better deal with such stresses by providing more of the raw materials needed for antioxidant enzyme synthesis. Thus, when CO_2-enriched sugar maple seedlings were subjected to an additional 200 ppb of ozone, Niewiadomska *et al.* (1999)[4] reported that ascorbate peroxidase, which is the first line of enzymatic defense against ozone, significantly increased. Likewise, Schwanz and Polle (2001)[5] noted that poplar clones grown at 700 ppm CO_2 exhibited a much greater increase in superoxide dismutase activity upon chilling induction than clones grown in ambient air. In addition, Lin and Wang (2002)[6] observed that activities of superoxide dismutase and catalase were much higher in CO_2-enriched wheat than in ambiently-grown wheat following the induction of water stress.

> *A 75% increase in atmospheric CO_2 concentration boosted the vitamin C concentration within the juice of sour oranges by 5% over a five-year period.*

In some cases, the additional carbon fixed during CO_2-enrichment is invested in antioxidative compounds, rather than enzymes. In the study of Idso *et al.* (2002)[7], for example, a 75% increase in atmospheric CO_2 concentration boosted the vitamin C concentration within the juice of sour oranges by 5% over a five-year period. In addition, Estiarte *et al.* (1999)[8] reported that a 180-ppm increase in the air's CO_2 content increased the foliar concentrations of flavonoids, which protect against UV-B radiation damage, in field-grown spring wheat by 11 to 14%. And it is interesting to note that the increased consumption of such plant material - naturally enriched with antioxidative compounds as a consequence of the historical rise in the air's CO_2 content - may have played a role in the observed decline in human mortality rates over the period 1950-1994 (Tuljapurkar *et al.*, 2000[9]).

A few years later, Wang *et al.* (2003)[10] evaluated the effects of elevated CO_2 on the antioxidant activity and flavonoid content of strawberry fruit that they grew out-of-doors in six clear-acrylic open-top chambers, two of which were maintained at the ambient atmospheric CO_2

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[4] http://www.co2science.org/articles/V2/N19/B5.php
[5] http://www.co2science.org/articles/V4/N11/B1.php
[6] http://www.co2science.org/articles/V5/N41/B1.php
[7] http://www.co2science.org/articles/V5/N32/B1.php
[8] http://www.co2science.org/articles/V3/N35/B2.php
[9] http://www.co2science.org/articles/V3/N13/B2.php
[10] http://www.co2science.org/articles/V6/N34/EDIT.php

concentration, two of which were maintained at ambient + 300 ppm CO_2, and two of which were maintained at ambient + 600 ppm CO_2 for a period of 28 months (from early spring of 1998 through June 2000). The fruits of their labor were harvested twice - "at the commercially ripe stage" in both 1999 and 2000 - after which they had them analyzed for a number of different antioxidant properties and flavonol contents.

Before reporting what they found, however, Wang *et al*. provided some background by noting that "strawberries are good sources of natural antioxidants (Heinonen *et al*., 1998)." They further reported that "in addition to the usual nutrients, such as vitamins and minerals, strawberries are also rich in anthocyanins, flavonoids, and phenolic acids," and that "strawberries have shown a remarkably high scavenging activity toward chemically-generated radicals, thus making them effective in inhibiting oxidation of human low-density lipoproteins (Heinonen *et al*., 1998)." And in this regard, they noted that previous studies (Wang and Jiao, 2000; Wang and Lin, 2000) had shown that "strawberries have high oxygen radical absorbance activity against peroxyl radicals, superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen." In their experiment, therefore, they were striving to see if atmospheric CO_2 enrichment could make a good thing even better.

So what did they find? They determined, first of all, that strawberries had higher concentrations of ascorbic acid (AsA) and glutathione (GSH) when grown under enriched CO_2 conditions. In going from ambient to +300 ppm and +600 ppm CO_2, for example, AsA concentrations rose by 10 and 13%, respectively, while GSH concentrations increased by 3 and 171%, respectively. They also learned that "an enriched CO_2 environment resulted in an increase in phenolic acid, flavonol, and anthocyanin contents of fruit." For nine different flavonoids, for example, there was a mean concentration increase of 55 ± 23% in going from the ambient atmospheric CO_2 concentration to +300 ppm CO_2, and a mean concentration increase of 112 ± 35% in going from ambient to +600 ppm CO_2. In addition, they discovered that the "high flavonol content was associated with high antioxidant activity." As for the significance of these findings, Wang *et al*. stated that "anthocyanins have been reported to help reduce damage caused by free radical activity, such as low-density lipoprotein oxidation, platelet aggregation, and endothelium-dependent vasodilation of arteries (Heinonen *et al*., 1998; Rice-Evans and Miller, 1996)."

In summarizing their findings, Wang *et al*. wrote that "strawberry fruit contain flavonoids with potent antioxidant properties, and under CO_2 enrichment conditions, increased the[ir] AsA, GSH, phenolic acid, flavonol, and anthocyanin concentrations," further noting that "plants grown under CO_2 enrichment conditions also had higher oxygen radical absorbance activity against [many types of oxygen] radicals in the fruit." Hence, they determined that atmospheric CO_2 enrichment truly *did* "make a good thing better."

In another study from the same year, Baczek-Kwinta and Koscielniak (2003)[11] grew two hybrid genotypes - KOC 9431 (chill-resistant) and K103xK85 (chill-sensitive) - from seed in air of either

[11] http://www.co2science.org/articles/V7/N5/B1.php

ambient (350 ppm) or elevated (700 ppm) CO_2 concentration (AC or EC, respectively), after which the plants were exposed to air of 7°C for eleven days and then recovered in ambient air of 20°C for one day. Throughout this period, a number of physiological and biochemical parameters were measured on the plants' third fully-expanded leaves; and the two researchers determined thereby that "EC inhibited chill-induced depression of net photosynthetic rate (PN), especially in leaves of chill-resistant genotype KOC 9431," which phenomenon "was distinct not only during chilling, but also during the recovery of plants at 20°C." In fact, they noted that "seedlings subjected to EC showed 4-fold higher PN when compared to AC plants," also determining that "EC diminished the rate of superoxide radical formation in leaves in comparison to the AC control." In addition, they indicated that "electrolyte leakage from the [leaf membrane] tissue, a parameter reflecting membrane injury, was significantly lower in samples of plants subjected to EC than AC." Last of all, they discovered that enrichment of the air with CO_2 successfully inhibited the decrease in the maximal quantum efficiency of photosystem 2, both after chilling and during the one-day recovery period. And lumping all of these positive effects of elevated CO_2 together, the two scientists concluded their paper by saying that "the increase in atmospheric CO_2 concentration seems to be one of the protective factors for maize grown in cold temperate regions."

Shifting gears just a bit, Yu, L. *et al*. (2004)[12] noted in the introduction to their study that reactive oxygen species (ROS) generated during cellular metabolism or peroxidation of lipids and proteins play a causative role in the pathogenesis of cancer and coronary heart disease (CHD), as demonstrated by Slaga *et al*. (1987), Frenkel (1992), Marnett (2000) and Zhao *et al*. (2000). However, and fortunately so, as noted by Yu, L. *et al*., "antioxidant treatments may terminate ROS attacks and reduce the risks of CHD and cancer, as well as other ROS-related diseases such as Parkinson's disease (Neff, 1997; Chung *et al*., 1999; Wong *et al*., 1999; Espin *et al*., 2000; Merken and Beecher, 2000)." And as a result, the four U.S. Colorado State University scientists stated that "developing functional foods rich in natural antioxidants may improve human nutrition and reduce the risks of ROS-associated health problems."

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In light of these observations, it is only natural to wonder how global warming might affect crop ROS scavenging activities and chelating capacities, the latter of which may inhibit radical-mediated oxidative chain reactions by stabilizing transition metals that are required to catalyze the formation of the first few radicals needed to initiate the radical reactions (Nawar, 1996). And in one of the few studies

[12] http://www.co2science.org/articles/V7/N20/B3.php

to broach this subject, Wang and Zheng (2001) examined the effects of a group of day/night temperature combinations on the antioxidant activities of the juice of two strawberry varieties, finding, in the words of Yu *et al*., that "the highest day/night temperature resulted in fruits with the greatest phenolic content as well as antioxidant activities." And thus encouraged by this finding, the Fort Collins scientists decided to explore the subject further in a study of winter wheat.

Very briefly, flour extracts of three hard winter wheat varieties that were grown at five different locations in Colorado were examined and compared for their radical scavenging properties, chelating capacities and total phenolic contents. Although no statistically significant correlations were found, the scientists said that "a correlation coefficient of 0.890 (P = 0.110) was detected for the chelating activity of Akron flour and the total hours of the growth location exceeding 32°C during the 6-week grain-filling period." Thus, although no firm conclusions could be drawn from the results of their study, in contrast to the study of Wang and Zheng (2001), Yu *et al*.'s findings were intriguing enough to lead them to state that "more research is needed to clarify how varieties and growing conditions alter the antioxidant properties of wheat, wheat flour and bran," for this is a subject of enormous importance that has been largely neglected in the global warming debate of the past several years; and it is a deficiency that must be remedied.

Publishing concurrently were Lu, J. *et al*. (2004)[13], who wrote that "oxidative stress is potentially experienced by all aerobic life when exposed to UV-B radiation," and that "elevated CO_2 can enhance the capacity of plants to resist stress-induced oxidative damage," citing the study of Ren *et al*. (2001) who worked with *terrestrial* plants. Hence, they set about to see if this was also the case with *marine* plants by focusing their attention on phytoplankton, which they described as "the single most important ecosystem on our planet."

In this effort, the marine microalgae *Platymonas subcordiformis* (Wille) Hazen was grown in the laboratory at ambient levels of atmospheric CO_2 concentration and UV-B radiation flux density, as well as at elevated levels of 5000 ppm CO_2 and UV-B radiation characteristic of that anticipated to result from a 25% stratospheric ozone depletion under clear sky conditions in summer. By itself, and by these means, the five researchers determined that the elevated UV-B treatment significantly *decreased* microalgal dry weight, photosynthetic rate, chlorophyll *a* and carotenoid contents, while the elevated CO_2 treatment by itself *enhanced* dry weight and photosynthetic rate, while chlorophyll *a* content and carotenoid content exhibited no major differences compared with those of ambient UV-B and ambient CO_2. They also reported that elevated UV-B by itself significantly increased the production of the toxic superoxide anion and hydrogen peroxide, as well as malonyldialdehyde, which is an end product of lipid peroxidation, whereas elevated CO_2 by itself did just the opposite. In addition, in the treatment consisting of both elevated UV-B and elevated CO_2, the concentrations of these three substances were lower than those observed in the elevated UV-B and ambient CO_2 treatment. Finally, they found that

[13] http://www.co2science.org/articles/V7/N42/B3.php

elevated CO_2 decreased the levels of several antioxidative enzymes found in the microalgae, reflective of their reduced need for detoxification of reactive oxygen species in the elevated CO_2 treatment.

In discussing the implications of their several findings, Yu *et al*. wrote that their results suggest that "CO_2 enrichment could reduce oxidative stress of reactive oxygen species to *P. subcordiformis*, and reduce the lipid peroxidation damage of UV-B to *P. subcordiformis*." They also said that "CO_2 enrichment showed a protective effect against the oxidative damage of UV-B-induced stress," and, therefore, that "elevated CO_2 can be [in] favor of enhancing the capacity of stress resistance." Put more simply, they stated in their concluding paragraph that "we have shown that algae grown under high CO_2 would better overcome the adverse impact of environmental stress factor[s] that act via generation of activated oxygen species."

One year later, Caldwell *et al*. (2005)[14] wrote that "the beneficial effects of isoflavone-rich foods have been the subject of numerous studies," specifically citing Messina (1999) and Birt *et al*. (2001), while adding that "foods derived from soybeans are generally considered to provide both specific and general health benefits," presumably via these substances. Hence, it was only natural that they should wonder how the isoflavone content of soybean seeds might be affected by the ongoing rise in the air's CO_2 content, and that they would thus conduct a set of experiments to find the answer.

In their study, well-watered and fertilized soybean plants were grown from seed to maturity in pots within two controlled-environment chambers, one maintained at an atmospheric CO_2 concentration of 400 ppm and one at 700 ppm. The chambers were initially kept at a constant air temperature of 25°C. At the onset of seed fill, however, air temperature was reduced to 18°C until seed development was complete, in order to simulate average outdoor temperatures at this stage of plant development. In a second experiment, this protocol was repeated, except that the temperature during seed fill was maintained at 23°C, with and without drought (a third treatment), while in a third experiment, seed-fill temperature was maintained at 28°C, with or without drought.

In the first experiment, where air temperature during seed fill was 18°C, the elevated CO_2 treatment increased the total isoflavone content of the soybean seeds by 8%. In the second experiment, where air temperature during seed fill was 23°C, the extra CO_2 increased total seed isoflavone content by 104%, while in the third experiment, where air temperature during seed fill was 28°C, the CO_2-induced isoflavone increase was 101%. And then, when drought-stress was added as a third environmental variable, the extra CO_2 boosted total seed isoflavone content by 186% when seed-fill air temperature was 23°C, while at a seed-fill temperature of 28°C, it increased isoflavone content by 38%.

[14] http://www.co2science.org/articles/V8/N21/B2.php

Therefore, under all of the environmental circumstances studied by Caldwell *et al*., enriching the air with an extra 300 ppm of CO_2 increased the total isoflavone content of soybean seeds. In addition, the percent increases measured under the stress situations investigated were always greater than the percent increase measured under optimal growing conditions. And thus it can be appreciated that the direct effects of atmospheric CO_2 enrichment on the health-promoting properties of soybean seeds are likely *universally beneficial* and a boon to the entire human race, especially in light of the fact that Bernacchi *et al*. (2005) have characterized the soybean as "the world's most important seed legume."

Moving on, plants cultured *in vitro* typically suffer from a number of physiological and biochemical impairments, such that upon transfer to *ex vitro* conditions they often experience severe oxidative stress. And, therefore, Carvalho *et al*. (2005)[15] conducted a study to see to what extent this stress might be alleviated by a nominal doubling of the air's CO_2 content. More specifically, they evaluated the damage done to the large subunit of Rubisco in grapevine (*Vitis vinifera* L.) plantlets while exposed to *in vitro* conditions and the degree to which that damage was ameliorated by atmospheric CO_2 enrichment during subsequent exposure to *ex vitro* conditions.

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In this study, the *in vitro* plantlet cultures were maintained in a growth chamber at a photon flux density (PFD) of 45 µmol m^{-2} s^{-1}, after which they were transferred to *ex vitro* conditions having a PFD of either 150 (low light) or 300 (high light) µmol m^{-2} s^{-1} and an air CO_2 concentration of either 350 (low CO_2) or 700 (high CO_2) ppm, while a number of physiological and biochemical measurements were made on the plantlets at seven-day intervals over a period of 28 days.

These efforts revealed that Rubisco degradation products were present in the leaves of plantlets in both *in vitro* and *ex vitro* conditions. However, as Carvalho *et al*. reported, "under low CO_2 they were maintained for almost all of the 28 days of the acclimatization period, while becoming scarcely detected after 14 days under high CO_2 and after 7 days when high CO_2 was associated with high light." In addition, they noted that "patterns of soluble sugars in acclimatizing leaves under high light and high CO_2 also gave an indication of a faster acquisition of autotrophic characteristics."

[15] http://www.co2science.org/articles/V8/N31/B2.php

Carvalho *et al*.'s results thus demonstrated the beneficial impact of high CO_2 concentrations in reducing the oxidative stress induced by the transfer of *in vitro*-produced plantlets to *ex vitro* conditions, as they stated that "a net benefit from high CO_2 treatments was clearly visible, contributing to an increased stability of Rubisco," adding that "the disappearance of Rubisco large subunit degradation products in persistent leaves subjected to the *ex vitro* treatments may be considered an indicator of recovery from stress."

Shifting from grapevines to ginseng plants (*Panax ginseng*) - the roots of which are widely cultivated in China, South Korea and Japan, and have been used for medicinal purposes since Greek and Roman times - we encounter another plant that is known for its anti-inflammatory, diuretic and sedative properties and is acknowledged to be an effective healing agent (Gillis, 1997; Ali *et al*., 2005). Normally, however, four to six *years* are required for ginseng roots to accumulate the amounts of the various phenolic compounds that are needed to produce their health-promoting effects. Consequently, in an important step in the quest to develop an efficient culture system for the commercial production of ginseng root, Ali *et al*. (2005)[16] investigated the effects of growing ginseng roots in suspension culture in bioreactors maintained in equilibrium with air enriched to CO_2 concentrations of 10,000 ppm, 25,000 ppm and 50,000 ppm for periods of up to 45 days.

Of most immediate concern in such an experiment would be the effects of the ultra-high CO_2 concentrations on root growth. Would they be toxic and lead to biomass reductions or even root death? The answer was a resounding *no*. After 45 days of growth at 10,000 ppm CO_2, for example, root dry weight was increased by fully 37% relative to the dry weight of roots produced in bioreactors in equilibrium with normal ambient air, while root dry mass was increased by a lesser 27% after 45 days at 25,000 ppm CO_2 and by a still smaller 9% after 45 days at 50,000 ppm CO_2. Hence, although the optimum CO_2 concentration for ginseng root growth clearly resided at some value lower than 10,000 ppm in this study, the concentration at which root growth rate was reduced below that characteristic of ambient air was somewhere significantly above 50,000-ppm, for even at that high CO_2 concentration, root growth was still greater than it was in ambient air.

Almost everything else measured by Ali *et al*. was even more dramatically enhanced by the ultra-high CO_2 concentrations they employed in their experiment. After 45 days of treatment, total root phenolic concentrations were 58% higher at 10,000 ppm CO_2 than at ambient CO_2, 153% higher at 25,000 ppm CO_2 and 105% higher at 50,000 ppm CO_2, as best as can be determined from the bar graphs of their results. Likewise, total root flavonoid concentrations were enhanced by 228%, 383% and 232%, respectively, at the same ultra-high CO_2 concentrations, while total protein contents rose by 14%, 22% and 30%, non-protein thiol contents by 12%, 43% and 62%, and cysteine contents by 27%, 65% and 100% under the identical respective set of conditions.

[16] http://www.co2science.org/articles/V8/N39/EDIT.php

What is more, there were equally large CO_2-induced increases in the activities of a large number of phenol biosynthetic enzymes.

What are the implications of these results? Ali *et al*. wrote that "the consumption of foodstuffs containing antioxidant phytonutrients such as flavonoids, polyphenolics, ascorbate, cysteine and non-protein thiol is advantageous for human health," citing Cervato *et al*. (2000) and Noctor and Foyer (1998). And, therefore, they concluded that their technique for the culture of ginseng roots in CO_2-enriched bioreactors could be used for the large-scale production of an important health-promoting product that could be provided to the public in much greater quantities than is currently possible.

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Also to be noted in this regard is the high probability that as the atmosphere's CO_2 concentration continues its *natural* upward climb, ginseng and many other medicinal plants will likely see the concentrations of their health-promoting carbon-based secondary compounds *naturally* increase, leading to better human health the world over. In fact, this phenomenon has likely already played a role (the magnitude of which is yet to be determined) in the huge lengthening of human life span that occurred over the course of the Industrial Revolution, when the air's CO_2 concentration rose from something on the order of 280 ppm to a value that is currently closing in on 400 ppm.

It is well known, for example, that *reactive oxygen species* (ROS) generated during cellular metabolism or peroxidation of lipids and proteins play a causative role in the pathogenesis of cancer and coronary heart disease (CHD), as demonstrated by Slaga *et al*. (1987), Frenkel (1992), Marnett (2000) and Zhao *et al*. (2000). However, Yu, L. *et al*. (2004) have noted that "antioxidant treatments may terminate ROS attacks and reduce the risks of CHD and cancer, as well as other ROS-related diseases such as Parkinson's disease (Neff, 1997; Chung *et al*., 1999; Wong *et al*., 1999; Espin *et al*., 2000; Merken and Beecher, 2000)," and they have therefore stated that "developing functional foods rich in natural antioxidants may improve human nutrition and reduce the risks of ROS-associated health problems."

Much the same point of view was expressed by Wilcox *et al*. (2004)[17], who wrote that oxidative stress "has been related to cardiovascular disease, cancer, and other chronic diseases that account for a major portion of deaths today," and who went on to discuss the role that exogenous antioxidants play in controlling oxidation and to review the evidence for their roles in preventing disease.

The three nutrition experts began their discussion of this important subject by stating that "diet plays a vital role in the production of the antioxidant defense system by providing essential nutrient antioxidants such as vitamin E, C, and ß-carotene, other antioxidant plant phenols including flavanoids, and essential minerals that form important antioxidant enzymes." In addition, they noted that "epidemiological data generally indicate a benefit of consuming diets that are higher in antioxidant nutrients, specifically diets high in fruits and vegetables."

But isn't it much easier to obtain these antioxidants by simply popping a pill or two (or even three or four) into one's mouth? It certainly is; and millions of people do it daily. But is this approach as effective as obtaining needed antioxidants via the food one eats? Probably not. Willcox *et al*. reported, for example, that in many studies of antioxidant health benefits "it is not clear whether the benefit is derived from the specific nutrients under study or another food component having health benefits yet to be discovered," or that perhaps "there is a particular combination of antioxidant nutrients that provide protection." While some epidemiological studies, in their words, "appear to demonstrate clear associations, direct tests of the relationships with clinical trials have not yielded similar results." In fact, they say that "the most convincing evidence of antioxidant effect on cancer prevention involves feeding fruits and vegetables rather than individual antioxidants."

Moving on, some four years later, Levine *et al*. (2008)[18] grew well watered and fertilized wheat plants (*Triticum aestivum*, cv Yocoro roho) from seed in custom-designed root modules - "consisting of a porous tube embedded in Turface (1-2 mm particle size) substrate containing 5 g Osmocote time release fertilizer per liter" - which were housed in Plexiglas chambers maintained at atmospheric CO_2 concentrations of either 400, 1500 or 10,000 ppm for periods of 14, 21 and 28 days, while measuring a number of plant metabolic properties, along with the leaf concentrations of several *flavonoids* that are capable of scavenging ROS. And what did they learn?

The thirteen researchers reported that "elevated CO_2 promoted the accumulation of secondary metabolites (flavonoids) progressively to a greater extent as plants became mature." As best as can be determined from the bar graphs of their results, for example, the percentage increase in total wheat leaf flavonoid concentration in going from an atmospheric CO_2 concentration of 400 to 1500 ppm was 22%, 38% and 27% at 14, 21 and 28 days after planting, respectively, while in going from a CO_2 concentration of 400 to 10,000 ppm, the percentage increase in total flavonoid

[17] http://www.co2science.org/articles/V9/N6/EDIT.php
[18] http://www.co2science.org/articles/V12/N8/B2.php

concentration was 38%, 56% and 86%, respectively, at 14, 21 and 28 days after planting. And they found that "both elevated CO_2 levels resulted in an overall 25% increase in biomass over the control plants."

In addition to the potential for the types of benefits described above, the U.S., Japanese and German scientists wrote that "the increased accumulation of secondary metabolites in plants grown under elevated CO_2 may have implications regarding plant-herbivore interactions, decomposition rates for inedible biomass, and potential beneficial effects on plant tolerance to water stress (Idso, 1988) and cold stress (Solecka and Kacperska, 2003) due to their potentials for the scavenging of reactive oxygen species (ROS)."

Also with a paper appearing in the same year were Stutte *et al*. (2008)[19], who while studying *Scutellaria* plants - herbaceous perennials that possess numerous medicinal properties - wrote that they are "rich in physiologically active flavonoids that have a wide spectrum of pharmacological activity." And in regard to this fact, they said that leaf extracts of *Scutellaria barbata* had been found to be "limiting to the growth of cell lines associated with lung, liver, prostate, and brain tumors (Yin *et al*., 2004)," and that "extracts of *S. lateriflora* and the isolated flavonoids from the extracts have been shown to have antioxidant, anticancer, and antiviral properties (Awad *et al*., 2003)." Hence, it was only natural for them to wonder how the growth of these important plants - and their significant medicinal properties - might be affected by the ongoing rise in the air's CO_2 content.

In an attempt to shed further light on the subject, Stutte *et al*. conducted experiments with both *S. barbata* and *S. lateriflora*, where they measured effects of elevated atmospheric CO_2 (1200 and 3000 ppm vs. a control value of 400 ppm) on plant biomass production and plant concentrations of six bioactive flavonoids - apigenin, baicalin, baicalein, chrysin, scutellarein and wogonin - all of which substances, according to them, "have been reported to have anticancer and antiviral properties," as described in the review papers of Joshee *et al*. (2002) and Cole *et al*. (2007).

These experiments were conducted in a large step-in controlled-environment chamber that provided a consistent light quality, intensity and photoperiod to six small plant growth chambers that had "high-fidelity control of relative humidity, temperature, and CO_2 concentration," each of which chambers was also designed to monitor nutrient solution uptake by six individual plants that they grew from seed for a period of 49 days.

With respect to plant productivity, i.e., fresh and dry weight production, the three U.S. researchers determined that in the case of *S. barbata*, increasing the air's CO_2 concentration from 400 to 1200 ppm resulted in a 36% increase in shoot fresh weight and a 54% increase in shoot dry matter, with no further increases between 1200 and 3000 ppm CO_2. In the case of *S. lateriflora*, on the other hand, the corresponding increases in going from 400 to 1200 ppm CO_2

[19] http://www.co2science.org/articles/V12/N24/EDIT.php

were 62% and 44%, while in going all the way to 3000 ppm CO_2, the total increases were 122% and 70%, respectively.

In the case of *total flavonoid concentrations* in the plants' vegetative tissues, Stutte *et al*. found that in the case of *S. barbata*, "the combined concentration of the six flavonoids measured increased by 48% at 1200 and 81% at 3000 ppm CO_2," while in *S. lateriflora* they indicated that "the total flavonoid content increased by over 2.4 times at 1200 and 4.9 times at 3000 ppm CO_2." Thus, in consequence of the *compounding effect* of increases in both *plant biomass* and *flavonoid concentration*, the total flavonoid content in *S. barbata* rose by 72% in going from 400 to 1200 ppm CO_2, and by 128% in going all the way to 3000 ppm CO_2, while in *S. lateriflora* the corresponding increases were a huge 320% and a mind-boggling 1,270%.

In the concluding sentence of their paper's abstract, Stutte *et al*. thus wrote that their results indicated that "the yield and pharmaceutical quality of *Scutellaria* species can be enhanced with controlled environment production and CO_2 enrichment," and *massively so* it would appear. In addition, since they indicated that over 200 substances - of which over 80% are flavonoids - have been found in a total of 65 *Scutellaria* species, it would also appear that the "increased concentration of flavonoids through CO_2 enrichment," as they concluded, "has the potential to enhance the production and quality of medicinal plants."

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One year later, Perez-Lopez *et al*. (2009)[20] introduced their study of oxidative stress by stating that *soil salinity* "is one of the major environmental constraints limiting plant productivity and distribution," affecting, as it does, "19.5% of the world's irrigated area," as well as "non-irrigated croplands and rangelands." And seeking to learn more about the subject, they grew two barley (*Hordeum vulgare* L.) cultivars, Alpha and Iranis, within controlled-environment growth chambers at either ambient (350 ppm) or elevated (700 ppm) atmospheric CO_2 concentrations in a 3:1 perlite:vermiculite mixture that was watered with Hoagland's solution every two days (until the first leaf was completely expanded at 14 days), after which a salinity treatment was administered by adding 0, 80, 160 or 240 m*M* NaCl to the Hoagland's solution every two days for 14 more days. Then, after a total of 28 days, the primary leaf of each barley plant was harvested and assessed for a number of biochemical properties.

[20] http://www.co2science.org/articles/V12/N9/B3.php

This work revealed that in the various ambient-air salinity treatments, the deleterious effects of reactive oxygen species (ROS) on barley leaves were made apparent through ion leakage and increases in *thiobarbituric acid reactive substances* (TBARS), which rose ever higher as salt concentrations rose ever higher. "On the other hand," as they continued, "when [the] salinity treatment was imposed under elevated CO_2 conditions, lower solute leakage and TBARS levels were observed, suggesting that the oxidative stress caused by salinity was lower."

In thus interpreting their findings, Perez-Lopez *et al*. said "it is concluded that elevated CO_2 protects barley cultivars from oxidative stress," noting that "the relief of oxidative stress damage observed in our barley leaves grown under a CO_2 enriched atmosphere has also been observed in alfalfa (Sgherri *et al*., 1998), pine (Vu *et al*., 1999) and oak (Schwanz and Polle, 2001b)." Hence, it would appear that the ongoing rise in the air's CO_2 content may help a wide variety of earth's plants to better cope with the many serious problems caused by high soil salinity.

In introducing another study of wheat, Varga *et al*. (2012)[21] wrote that "as well as damaging numerous physiological functions, abiotic stress [such as drought] also leads to higher concentrations of reactive oxygen species, which are present in nature in all plants, but which may damage cell components and disturb metabolic processes when present in larger quantities," citing the work of Omran (1980), Larson (1988) and Dat *et al*. (2000). On the other hand, they indicated that "many authors have demonstrated that the [atmosphere's] CO_2 concentration has a substantial influence on the stress sensitivity of plants via changes in antioxidant enzyme activity," citing Fernandez-Trujillo *et al*. (2007), Ali *et al*. (2008) and Varga and Bencze (2009), so that increases in the atmosphere's CO_2 concentration may increase various plant antioxidant enzymes and thereby *reduce* the negative effects of various abiotic stresses.

Enhanced CO_2 concentration "delayed the development of drought stress up to first node appearance, and stimulated antioxidant enzyme activity when drought occurred during ripening, thus reducing the unfavorable effects of [drought] stress."

In an experiment designed to further explore this subject, Varga *et al*. grew two varieties of winter wheat within phytotrons maintained at either 380 or 750 ppm CO_2, where the potted plants were watered daily and supplied with nutrient solution twice a week until the start of drought treatments, when drought was induced in three phases - at first node appearance, heading and grain filling - by completely withholding water for seven

[21] http://www.co2science.org/articles/V15/N38/B3.php

days, which dropped the volumetric soil water content in the pots from 20-25% to 3-5%. And in terms of what they learned, the four researchers - all of whom were associated with the Agricultural Research Institute of the Hungarian Academy of Sciences - reported that they observed "changes in enzyme activity" that "indicated that enhanced CO_2 concentration delayed the development of drought stress up to first node appearance, and stimulated antioxidant enzyme activity when drought occurred during ripening, thus reducing the unfavorable effects of [drought] stress," and leading Varga *et al*. to conclude that the increases in the antioxidant enzymes they analyzed "may help to neutralize the reactive oxygen species induced by stress during various parts of the vegetation period," which phenomenon may help mankind's crops to better cope with whatever extremes of moisture insufficiency might be lurking in our future.

Rounding out this particular summary, Farfan-Vignolo and Asard (2012)[22] began the report of their research by noting that "grassland communities constitute an important fraction of the green surface of the earth, and are worldwide an important source of cattle-food (Carlier *et al*., 2009; Ciais *et al*., 2011)." And in light of these facts, the pair of Belgian researchers investigated several physiological and molecular (antioxidant) responses to water deficit in two major grassland species (*Lolium perenne* L. and *Medicago lupulina* L.) under current *ambient* (A) and future *elevated* (E) atmospheric CO_2 concentrations and air temperatures (T), where $ECO_2 = ACO_2 + 375$ ppm, and where ET = AT + 3°C.

"Not surprisingly," as they described it, "drought caused significant increases in oxidative damage, i.e., in protein oxidation and lipid peroxidation levels." But they found that "in both species the impact of drought on protein oxidation was reduced in future climate conditions [ECO_2 and ET]." And speaking of the stress-reducing effect of ECO_2, they said that "this 'CO_2-protection effect' is reported for a variety of abiotic stress conditions and species," citing the studies of Schwanz and Polle (1998), Sgherri *et al*. (2000), Geissler *et al*. (2009), Perez-Lopez *et al*. (2009), Vurro *et al*. (2009) and Salazar-Parra *et al*. (2012), after which they indicated that they "find support for this effect at the level of oxidative cell damage and protein oxidation in water-deficit responses of *L. perenne* and *M. lupulina*." And, of course, they also found that *even under drought stress*, "elevated CO_2 significantly affected shoot production in *L. perenne* (increase by 27-32%)," and that "also in *M. lupulina* a biomass increase was observed (26-38%)."

In summing up the primary findings of *all* of the studies described herein, it is apparent that when oxidative stresses are present, elevated CO_2 helps to increase the synthesis and activities of antioxidants that tend to alleviate the various problems caused by the stresses. In addition, the possibility exists that the greater concentrations of antioxidant compounds in plant tissues caused by the historical rise in the air's CO_2 content may have contributed to the increase in human life span experienced over the course of the Industrial Revolution.

[22] http://www.co2science.org/articles/V16/N6/B1.php

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