How Terrestrial Organisms Sense, Signal, and Respond to Carbon Dioxide*

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SYNOPSIS. Because of anthropogenic increases in atmospheric CO₂ content, there is a need to understand how organisms sense and respond to CO₂ variation. An important distinction is whether CO₂ responses result from direct effects of CO₂ on signal-transduction pathways, enzyme catalysis, or regulatory processes, as opposed to indirect, secondary responses that are a consequence of the direct effects. In plants, direct effects occur because rising CO₂: A) increases the activity of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) via its role as a substrate for RuBP carboxylation and its inhibition of RuBP oxygenation; B) reduces stomatal aperture; C) alters mitochondrial respiration; and D) possibly reduces transcription of genes for Rubisco activase and carbonic anhydrase. Because of these direct effects, the carbon and water balance of plants is altered leading to secondary effects on growth, resource partitioning and defense compound synthesis. Reduced investment in photosynthetic protein is one of the characteristic acclimation responses of plants to high CO₂. This is modulated by increased carbohydrate levels, probably in concert with hormone signals from the roots. Roots are hypothesized to be the main control points for CO₂ acclimation because they are well situated to integrate the carbohydrate status of the plant. In higher fungi, development of the mushroom fruiting body is inhibited at high CO₂, but the mechanism is poorly known. Fungal CO₂ sensing may serve to position the spore-bearing tissue above the soil boundary layer to ensure effective spore dispersal. The animals that are most sensitive to anthropogenic CO₂ enrichment are insects. Many insects have a well-developed ability to sense CO₂ variation as a means of locating food. Unlike plants, insects have CO₂ receptors that can detect variation in CO₂ as low as 0.5 ppm. However, the sensitivity of these receptors is reduced in atmospheres with double or triple current levels of CO₂, indicating some insect species may be threatened by rising atmospheric CO₂.

INTRODUCTION

Because of fossil fuel use and deforestation, humans have increased the CO₂ level in the atmosphere from 270 ppm two hundred years ago to 370 ppm today. In the coming centuries, atmospheric CO₂ levels are expected to double, and may triple before peaking. The human effect on atmospheric CO₂ content is super-imposed upon natural CO₂ variations controlled by long-term changes in the global carbon cycle. Over the past 400,000 yr, atmospheric CO₂ has oscillated between 180 and 300 ppm in concert with glacial/interglacial cycles (Petit et al., 1999). During interglacial periods, CO₂ levels peaked near 300 ppm for about 10,000 to 15,000 yr. During glacial phases, low CO₂ levels persisted for much longer. CO₂ levels were below 240 ppm for two-thirds of the past 400,000 yr and over 80,000 yr of this period corresponded to CO₂ levels below 200 ppm (Petit et al., 1999; Sage and Coleman, 2001). Because of these generally low CO₂ levels of the past half-million years, plants are probably adapted to much lower CO₂ than exists today (Sage and Coleman, 2001).

In addition to the natural cycles of recent geological time, a long-term decline in atmospheric CO₂ has occurred over the past 100 million years, from levels estimated to be 3 to 10 times above current levels (Berner, 1994). Associated with this decline has been a general cooling and drying of the global climate, and an extensive radiation of plant and animal life forms (Prothero, 1994). For example, most angiosperm families appeared during the time when CO₂ declined from the high levels that existed during the time of the dinosaurs (Wolfe, 1997). This reduction in CO₂ has been implicated as a contributing agent for numerous evolutionary trends, notably the rise of plants that use the C₄ photosynthetic pathway, and evolutionary specialization of numerous faunal lines (Ehleringer et al., 1991, 1997; MacFadden, 1997; Sage, 1999). In addition, numerous water conservation features in plants may have arisen as CO₂ levels fell, because of the reduced water use efficiency that accompanies reduction in atmospheric CO₂ (Sage and Cowling, 1999).

Over the years, the focus of CO₂-related studies has been the response of plants to rising CO₂, largely because of their ability to acquire CO₂ through photosynthesis. It is now recognized that a wide variety of organisms directly respond to CO₂ variation, and plants are not necessarily the most responsive, nor are they the most threatened, by atmospheric CO₂ enrichment. Atmospheric CO₂ variation affects organisms through direct interactions with enzymes, sensory molecules, or regulatory systems, and indirectly as a result of secondary responses to these direct effects. To understand the range of effects of CO₂, it is necessary to identify the responses that are direct, versus those that are secondary in nature, or indirect. The purpose of this paper is to review our understanding of the mechanisms by which organisms directly respond to variation in CO₂, and how these responses cascade to affect higher order processes within an organism.
Effects of Atmospheric CO₂ Variation on Plants

The direct effects of rising atmospheric CO₂ on plants are due to three major processes: a) direct modulation of the activity of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the primary CO₂ fixing enzyme in photosynthesis, b) sensing of CO₂ levels by stomatal guard cells, and c) modulation of mitochondrial respiration (Fig. 1; Sage and Reid, 1994; Drake et al., 1997). The consequence of each of these direct effects is to alter water and carbohydrate levels in plants, which in turn modulate a diverse set of secondary responses ranging from stimulation of growth, alteration of biomass allocation, increased chemical defense, and greater density of mycorrhizal and nitrogen fixing associations (Sage, 1995; Lindroth, 1996; Drake et al., 1997; Rogers et al., 1999). Direct sensing of CO₂ has been suggested to control expression of photosynthetic genes coding for carbonic anhydrase and Rubisco activase (Fig. 1; Coleman, 2000; von Caemmerer and Quick, 2000), but this control is relatively minor and does not have a major effect on photosynthetic performance at CO₂ levels near the current ambient and above. Instead, the principal mechanism by which photosynthesis responds to CO₂ is via the direct stimulation of Rubisco activity.

The initial reaction in photosynthetic CO₂ fixation is the carboxylation of RuBP; however, Rubisco also can oxygenate RuBP in the first step of a process termed photorespiration (Fig. 2; Jordan and Ogren, 1984). Oxygenation of RuBP produces phosphoglycolate, a useless two-carbon compound. Metabolism of phosphoglycolate to recover its carbon requires ATP and reducing power, and results in the loss of previously fixed CO₂ at a ratio of 1 C lost for every two oxygenation events (Sharkey, 1988). The uptake of oxygen via RuBP oxygenation, and the loss of CO₂ during phosphoglycolate metabolism is termed photorespiration because of its superficial similarity with mitochondrial respiration. CO₂ and O₂ compete for Rubisco active sites, and thus the ratio of photorespiration to photosynthesis is highly dependent on the ratio of CO₂ to O₂ in the chloroplast (Fig. 3; Sage, 1999). In terrestrial plants, Rubisco has a much higher specificity for CO₂ relative to O₂, but atmospheric O₂ levels are 580 times higher than CO₂ in the current atmosphere, and over 1,000 times higher in the atmosphere.
of the late-Pleistocene. This abundance of O_2 relative to CO_2 compensates for the greater CO_2 specificity of Rubisco such that at warmer temperatures, photorespiration can be significant in atmospheres of today and recent geological time. Rising temperature reduces both the specificity of Rubisco for CO_2, and the CO_2/O_2 ratio in the chloroplast solution (Jordan and Ogren, 1984). As a result, photorespiration rises with increasing temperature, except at very high CO_2 levels (>1,000 ppm) where oxygenase activity is largely suppressed at all temperatures (Fig. 3). In large part because of the temperature dependence of photorespiration, the proportional stimulation of photosynthesis by rising CO_2 increases with temperature (Fig. 4). As a result, the CO_2 effect on the earth’s flora is predicted to be greatest in warmer climates of low latitude, and during the warmest peaks of the growing season at temperate latitude (Long, 1991).

At the molecular level, CO_2 has not been shown to directly interact with any receptor or step in the signal-transduction pathway of photosynthetic genes. Limited evidence has been presented that elevated CO_2 inhibits the transcription of genes coding for carbonic anhydrase and Rubisco activase (Majeau and Coleman, 1996; Mate et al., 1996; Eckard et al., 1997). It is not clear whether these responses are directly mediated by CO_2, or indirectly controlled by other factors, for example, carbohydrate status in the leaf (Coleman, 2000). Both enzymes enhance photosynthesis at low CO_2 levels but are less important at elevated CO_2. Carbonic anhydrase reversibly catalyzes the conversion of CO_2 to bicarbonate and in doing so can speed the diffusion of inorganic carbon into the chloroplast (Coleman, 2000). Rubisco activase enhances the activation state of Rubisco by removing inhibitors from the catalytic site (Portis, 1992). At low CO_2, plants are unable to maintain a high activation state of Rubisco in the absence of an abundant amount of Rubisco activase, while at elevated CO_2, less activase is required for complete Rubisco activation (Mate et al., 1996; Eckard et al., 1997).

SECONDARY RESPONSES OF THE PHOTOSYNTHETIC APPARATUS

Short-term responses

In C_3 plants, photosynthesis is limited by one of three general processes: the capacity of Rubisco to consume RuBP, the capacity of electron transport and the Calvin cycle reactions to regenerate RuBP, and the capacity of starch and sucrose synthesis to regenerate phosphate for photophosphorylation (Sharkey, 1985; Sage, 1994). The degree to which these processes limit photosynthesis varies with CO_2 level. At low CO_2 (370 ppm ambient CO_2 and below), Rubisco capacity exerts greater control over photosynthesis; at elevated CO_2 processes contributing to either RuBP or P, regeneration are limiting (Harley and Sharkey, 1991). As a result, a shift in CO_2 level produces a metabolic imbalance between the capacities of the different components of photosynthesis. This leads to a regulatory response that modulates activation states of key enzymes, but does not initially alter levels of existing enzyme (Sage, 1990). For example, tripling of atmospheric CO_2 causes Rubisco capacity to become excessive, and the response within the leaf is to reduce the activation state of Rubisco (Sage et al., 1988, 1989). Similarly, CO_2 reduction leads to a limitation of Rubisco capacity, and an excess of light harvesting and sucrose synthesis capacity (Sharkey et al., 1988; Sage, 1990; Sage et al., 1990). Regulation within the leaf reduces the capacity of light harvesting in CO_2-depleted atmospheres by activating carotenoid systems that quench excess light energy, while excess sucrose synthesis capacity is reduced by deactivation of sucrose-phosphate synthase and cytosolic fructose-bisphosphatase (Sharkey et al., 1988; Sage and Reid,
1994). These regulatory responses can be profound; for example, half of the Rubisco active sites can be switched off by large CO2 increases (Sage et al., 1988, 1989). They are, however, not directly related to CO2 acting as a regulatory molecule. Instead, the regulatory system responds to imbalances in the supply and demand for energy which CO2 variation perturbs through its effect on the carboxylation capacity of Rubisco (Sage, 1990; von Caemmerer and Quick, 2000).

Long-term regulation (photosynthetic CO2 acclimation)

The photosynthetic stimulation that initially follows high CO2 exposure generally leads to a significant enhancement of leaf carbohydrate levels and a burst of growth (Jitla et al., 1997; Centritto et al., 1999). Within a few days to weeks of CO2 enhancement, leaf protein levels may begin to decline, photosynthetic capacity at a given CO2 concentration drops, and the growth enhancement is reduced if not eliminated altogether (Stitt, 1991; Moore et al., 1997, 1999). These changes are also coupled to increases in root growth relative to shoot growth such that root to shoot ratios increase (Rogers et al., 1999). Together, these responses are termed the acclimation response to rising CO2. Much work has recently focused on understanding the signal-transduction system that controls the acclimation response.

The strength of the acclimation response is dependent upon nutrient status, plant age, photoperiod and source to sink ratio (Arp, 1991; Stitt, 1991; Roitsch, 1999; Stitt and Krapp, 1999). Low nutrients, advanced age, extended photoperiod, and sink loss all enhance the degree to which plants acclimate to CO2 enrichment. For example, older plants with low nutrient supply and few sinks often acclimate to the point where photosynthesis and growth enhancements disappear (Arp, 1991; Sage, 1994; Sims et al., 1998a). By contrast, young, rapidly growing plants with high nutrition and abundant sinks typically show little if any acclimation to rising CO2 (Sage, 1994; Drake et al., 1997; Sage and Coleman, 2001). Notably, many environmental factors that perturb nutrient supply and source to sink ratios in the absence of CO2 variation promote the same acclimation responses that occurs in elevated CO2 (Stitt, 1991). A common element in each of these responses is an enhancement of leaf carbohydrate content, and it is now recognized that hyper-accumulation of carbohydrates is the major signal controlling plant acclimation to elevated CO2, as well as acclimation to a variety of treatments that alter source to sink ratios (Stitt, 1991; Smeekens and Rook, 1997; Moore et al., 1999).

Carbohydrate signaling in photosynthetic acclimation

In leaves, most of the major photosynthetic genes respond to carbohydrate status. When carbohydrate levels are high, transcription of genes coding for Rubisco, chlorophyll-protein complexes of the light harvesting antennae, and chloroplast electron transport proteins is repressed (Moore et al., 1997, 1999). In sink tissues, multiple genes coding for the utilization and storage of carbohydrate are expressed, leading to enhanced activity of growth and storage tissues (Koch, 1996). The means by which tissues sense variation in carbohydrate status remains unclear, although multiple sensory pathways are postulated. The most discussed model of sugar sensing is hexokinase signaling. Hexoses (glucose and fructose) appear to be major activators of regulatory hexokinases in a manner that has not yet been identified (Jang et al., 1997; Smeekens, 2000). Once activated, hexokinase is hypothesized to initiate a signal-transduction sequence involving a number of intermediate complexes (termed SNF complexes for sucrose non fermenting after the mutant yeast in which they were first identified), that modulate transcription of specific genes. Hexokinase signaling in plants is similar to hexokinase sugar-sensing in animal and fungi, because regulatory hexokinases in each have high sequence similarity and the intermediate SNF complexes appear homologous (Jang et al., 1997; Halford and Hardie, 1998; Smeekens, 2000). Key differences are present, however. In animal and fungi, the primary purpose of hexokinase signaling is to coordinate sugar metabolism with availability of carbohydrates in the diet, and thus to avoid wasteful expression of enzymes if carbohydrates are deficient. In plants, the situation is more complex in that carbohydrate signals are required to coordinate protein investment within photosynthetic cells with metabolism in remotely located sinks, as well as transport between those sinks and local storage pools (Moore and Sheen, 1999). To accomplish this, plants have multiple sugar sensing mechanisms in addition to the hexokinase system. Sucrose, for example, directly modulates transcription of sucrose transporters (Smeekens and Rook, 1997; Chio and Bush, 1998). Membrane-bound receptors for specific carbohydrates are postulated, and acetate levels may have an important signaling function (Koch et al., 2000). Invertase activity is correlated with high CO2 acclimation (Moore et al., 1998), indicating an important role for this enzyme in sugar signaling. Moore et al. (1999) postulate that invertases hydrolyze excess sucrose to glucose and fructose, which are then rephosphorylated by hexokinase, leading in turn to hexokinase activation and signal production. In this manner, sucrose levels could be linked to the hexokinase regulatory system.

Interaction with phytohormones, nutrients and light

While much of the research on CO2 acclimation has focused upon the role of carbohydrates, it is increasingly clear that in plants, carbohydrates status is but one component of a complex interaction of controls that include phytohormones, light and nutrients (Roitsch, 1999; Smeekens, 2000). Carbohydrates alone cannot explain the range of acclimation responses to elevated CO2 as has been demonstrated by Sims et al. (1998b) using soybean plants grown in either normal or CO2-enriched atmospheres (Table 1). In their experiments, plants were grown in growth chambers in
atmospheres of either 250 or 1,000 ppm CO₂. From each plant, a single, attached leaf was placed in a smaller chamber and grown for ten days at either 250 or 1,000 ppm CO₂. Single leaves grown at 1,000 ppm showed little sign of photosynthetic acclimation when attached to plants growing at 250 ppm, because they had similar Rubisco levels as leaves exposed to 250 ppm on plants grown at 250 ppm (Table 1). By contrast, leaves in 250 ppm CO₂, but attached to plants at 1,000 ppm, showed marked acclimation, with 60% reduction in Rubisco levels, similar to what the high-CO₂-exposed leaves on high-CO₂-grown plants exhibited. Notably, carbohydrate levels did not correlate with the degree of acclimation, and tended to be lower in leaves of the high-CO₂ grown plants. As demonstrated by Sims et al. (1998b), entire plants, not simply leaves, acclimate to high CO₂.

Where acclimation within a leaf is mediated by whole-plant factors, long-distance signals molecules are likely involved. In plants, the major long-distance signals include the phytohormones (ABA, ethylene, gibberellins, auxin and cytokinins), xylem pH, and the nutrients nitrogen and calcium (Jackson, 1993; Davies et al., 1994; Davies and Gowing, 1999). High ABA in leaves may act in concert with ethylene and sucrose to modulate nutrient levels in leaves and antagonize senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf senescence, and thus may counteract the tendency of elevated carbohydrates to accelerate leaf sens}

<table>
<thead>
<tr>
<th>Condition</th>
<th>Glucose mg m⁻²</th>
<th>Fructose mg m⁻²</th>
<th>Sucrose mg m⁻²</th>
<th>Starch g m⁻²</th>
<th>Rubisco μmol m⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf at 250 ppm</td>
<td>88 ± 5</td>
<td>57 ± 5</td>
<td>417 ± 59</td>
<td>15 ± 2</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Plant at 250 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf at 1,000 ppm</td>
<td>138 ± 28</td>
<td>90 ± 15</td>
<td>654 ± 121</td>
<td>31 ± 4</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>Plant at 250 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf at 250 ppm</td>
<td>60 ± 3</td>
<td>38 ± 1</td>
<td>153 ± 21</td>
<td>12 ± 2</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Plant at 1,000 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf at 1,000 ppm</td>
<td>87 ± 9</td>
<td>53 ± 7</td>
<td>276 ± 44</td>
<td>28 ± 1</td>
<td>6 ± 0.3</td>
</tr>
<tr>
<td>Plant at 1,000 ppm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

* Rubisco units are μmol of catalytic sites per m⁻² of leaf tissue. Developed from Sims et al. (1998b) using results kindly supplied by Dan Sims. Means ± SE.

The second major direct effect of CO₂ is upon stomatal aperture. As atmospheric CO₂ levels increase, stomatal conductance (an index of stomatal aperture) declines, reducing transpiration and increasing the water use efficiency of photosynthesis (Morison, 1987; Mott, 1990). Drought stress and low atmospheric humidity enhance the degree of stomatal closure that is caused by CO₂ enrichment, so that the CO₂ effect on stomatal aperture is greatest in arid conditions.
The "stop-go" model of carbohydrate sensitivity in leaves, where root signals poise a leaf to have high (the stop mode) or low (the go mode) sensitivity to carbohydrates. In roots with low carbohydrate levels, ABA synthesis may be low, while cytokinin (CK) synthesis and release may be high. Cytokinin may then travel to leaves where it antagonizes carbohydrates signals and any effect of endogenous ABA that may be present. As a result, photosynthetic gene transcription may continue in the go-mode. In high carbohydrate roots, ABA synthesis and release may be pronounced, and once this reaches the leaf, may enhance the strength of a given carbohydrate signal, thereby reducing or stopping photosynthetic gene transcription. Nutrients may modify the response at both the leaf and root level, with proportionally high nutrients in the roots favoring cytokinin release, while proportionally low nutrients favor ABA production. (Schulze et al., 1987).

Despite this long-standing knowledge of stomatal responses to CO₂, the means by which CO₂ is sensed by guard cells remains uncertain. Guard cells do have functional chloroplasts, indicating internal photosynthetic activity could be a means by which guard cells sense variation in CO₂. Significantly, however, stomata respond to CO₂ enrichment in the dark when photosynthesis is not operating, indicating there is a direct sensing capability (Morison, 1987). Four models have been proposed to explain CO₂ sensing by stomata (Assmann, 1999). Two involve malate formation as a result of increased PEP carboxylation by PEP carboxylase. Rashke (1979) proposed elevated malate levels reduce cellular pH and the permeability of the tonoplast and plasmalemma of guard cells. In support of this model, reduction in cytosolic pH of guard cells by elevated levels of organic acids activates K⁺-efflux (Rashke, 1979). Hedrich and co-workers (Hedrich et al., 1994) argue that increased malate production enhances extracellular malate levels, which in turn activate anion efflux channels at the plasmalemma. As a result, osmotically-active anions leak out of the cell, causing the cell’s turgor pressure to decline, closing the stomata. Instead of malate, elevated zeaxanthin levels within the chloroplast may modulate guard cell CO₂-sensitivity in the light. Zeaxanthin content in guard cells is correlated with CO₂ level, but how this shift is transduced to the plasma-membrane is not known (Zhu et al., 1998). The fourth model involves CO₂ induced changes in cytosolic calcium levels, possibly in a synergistic manner with ABA. This final model is the only model that may involve a specific CO₂ receptor, which upon interacting with CO₂ would send a signal that could feed into signal-transduction pathways mediated by intracellular calcium (Assmann, 1999). Finally, multiple modes of CO₂ perception may exist, raising the possibility that more than one of these models are correct. Zeaxanthin signaling, for example, may be an additional means of CO₂-sensing as it only is present in the light, and thus could not account for dark CO₂ responses (Zhu et al., 1998).

Long-term responses

As with the photosynthetic apparatus, stomata can acclimate to long-term variation in CO₂ supply. CO₂ acclimation of stomata involves either a relative change in the aperture at the growth CO₂ level, or a change in the sensitivity to a given level of CO₂ variation (Santrucek and Sage, 1996). In Chenopodium album, growth at 750 ppm CO₂ resulted in a weakening of the stomatal response when measurement CO₂ levels were experimentally varied between 300 and 800 ppm (Fig. 6). The consequence of CO₂ acclimation by the stomata was a recovery of the ability of the guard cells to respond to CO₂ variation above growth CO₂ levels. In C. album plants grown at current atmospheric CO₂ levels, stomata were insensitive to increasing CO₂ levels above 750 ppm, but stomata of plants grown at 750 ppm could respond to CO₂ up to at least 1,200 ppm. In general, however, consistent responses of stomatal conductance to rising CO₂ are not apparent, and strong interactions with water status, leaf age, and humidity are present (Sage, 1994; Assmann, 1999). Despite this variation, it is clear that in most species, stomatal acclimation does not override the closing response, and transpiration rates remain depressed in high CO₂ environments (Johnson et al., 1993; Sage and Santrucek, 1996; Anderson et al., 1993).
The improvement in water status of the plant and soil associated with reduced stomatal aperture leads to a wide range of higher order responses that can have profound effects on overall performance, soil properties and competitive ability (Sage, 1995; Owensby et al., 1996).

Fungal CO₂ Sensing

Because they largely live in soil or the remains of other organisms where CO₂ levels are often very high (>1,000 ppm), fungal mycelia are not very sensitive to rising CO₂, except indirectly through alteration in carbohydrate supply provided by higher plants in parasitic or mutualistic associations (Cooke and Whipps, 1993; Rogers et al., 1999). By contrast, pileus (fruiting body, or the cap of the mushroom) development in numerous basidiomycete species is directly sensitive to moderate CO₂ enrichment. CO₂ levels above 1,000 ppm repress expansion of the pileus and stimulate stipe growth. Developed from Stamets (1993).

<table>
<thead>
<tr>
<th>Species</th>
<th>CO₂ Level, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pleurotus</em> umbratus</td>
<td>&lt;500</td>
</tr>
<tr>
<td><em>Hericium</em> erinaceum</td>
<td>500–1,000</td>
</tr>
<tr>
<td><em>Coprinus</em> comatus</td>
<td>500–1,000</td>
</tr>
<tr>
<td><em>Pleurotus</em> pulmonarius</td>
<td>400–800</td>
</tr>
<tr>
<td>Other <em>Pleurotus</em> species</td>
<td>&lt;1,000</td>
</tr>
<tr>
<td><em>Grifola</em> frondosa</td>
<td>&lt;1,000</td>
</tr>
<tr>
<td><em>Lentinula</em> edodes</td>
<td>&lt;1,000</td>
</tr>
<tr>
<td><em>Pholiota</em> nameko</td>
<td>800–1,200</td>
</tr>
<tr>
<td><em>Psilocybe</em> cyanescens</td>
<td>1,000–2,000</td>
</tr>
<tr>
<td><em>Hypholoma</em> ulmaria</td>
<td>600–1,500</td>
</tr>
<tr>
<td><em>Hypholoma</em> tessulatus</td>
<td>1,000–5,000</td>
</tr>
<tr>
<td><em>Volvariella</em> volvacea</td>
<td>1,000–5,000</td>
</tr>
<tr>
<td><em>Hypholoma</em> capsulatum</td>
<td>1,000–5,000</td>
</tr>
<tr>
<td><em>Ganoderma</em> lucidum</td>
<td>&lt;2,000</td>
</tr>
<tr>
<td><em>Agrocybe</em> aerigma</td>
<td>&lt;2,000</td>
</tr>
<tr>
<td><em>Auricularia</em> polytricha</td>
<td>2,000–5,000</td>
</tr>
<tr>
<td><em>Flammulina</em> velutipes</td>
<td>2,000–4,000</td>
</tr>
<tr>
<td><em>Morchella</em> angusticeps</td>
<td>&lt;5,000</td>
</tr>
</tbody>
</table>

* CO₂ levels that are greater than those presented inhibit pileus formation and stimulate stipe growth. Developed from Stamets (1993).
Figure 7. The response of mushroom development to atmospheric CO₂ content in oyster mushroom (Pleurotus ostreatus). As growth CO₂ increases, the mushroom cap (pileus) is reduced in size (top panel) and the stalk (stipe) elongates, reducing cap size relative to stipe length (bottom panel). Mushrooms grew on spawn blocks in fumigation chambers at 18° to 20°C. Each trial consisted of two replicated spawn blocks in separate fumigation containers. Each data point is the mean of the 10 largest mushrooms collected from a spawn block. (Sage, unpublished)

Table 3. Animal sensitivity to atmospheric CO₂*.  

<table>
<thead>
<tr>
<th>Response type</th>
<th>Examples</th>
<th>Δ [CO₂] for observed effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory suppression</td>
<td>All animals</td>
<td>&gt;50,000 ppm</td>
</tr>
<tr>
<td>Spireacle aperture</td>
<td>Insects</td>
<td>&gt;10,000 ppm</td>
</tr>
<tr>
<td>Olfactory sensation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematophagous parasites</td>
<td>mosquitoes, ticks, fire bugs, tsetse flies</td>
<td>&gt;10 to 500 ppm (activation), &gt;1,000 ppm (locomotion)</td>
</tr>
<tr>
<td>Social Insects</td>
<td>ants, bees, termites</td>
<td>&gt;5,000 ppm</td>
</tr>
<tr>
<td>Soil fauna</td>
<td>beetles, nematodes</td>
<td>&gt;5,000 ppm</td>
</tr>
<tr>
<td>Phytophagous insects</td>
<td>moths, butterflies</td>
<td>0.5 to 300 ppm</td>
</tr>
</tbody>
</table>

* Developed from Nicolas and Sillans (1989) and Stange (1996).
The organisms with the most acute sense of CO$_2$ perception are the lepidopteran herbivores (moths and butterflies) who can discriminate small changes (<1 ppm) in CO$_2$ brought about by photosynthetic activity of their plant foods (Stange, 1992, 1996, 1997). Caterpillars of the moth *Helicoverpa armigera* discriminate between CO$_2$ levels of 160 and 800 ppm, indicating an ability to distinguish between photosynthetic and respiring plant parts (Rasch and Rembold, 1994). This aids in locating fruit as opposed to leaf food supplies. In adult *Helicoverpa armigera*, CO$_2$ sensilla on labial palps are able to discriminate 0.5 ppm on a background of 350 ppm (Stange, 1992). The best-studied CO$_2$ sensing system in lepidopterans is that of the prickly-pear cactus moth, *Cactoblastis cactorum* (Stange et al., 1995; Stange, 1996, 1997). *Cactoblastis cactorum* locates its food supply by sensing nighttime reductions in CO$_2$ caused by the cladodes (leafy stems) of prickly pear cacti (*Opuntia* spp). *Opuntia* use CAM photosynthesis, which involves nighttime opening of the stomates and CO$_2$ fixation into a temporary pool of four-carbon acids. *Cactoblastis* species are important herbivores of *Opuntia* spp. and are well-known because they were introduced to control a severe *Opuntia* invasion in Australia early in the 20th century. CO$_2$ is sensed by *Cactoblastis* for two purposes. First, CO$_2$ gradients above the soil are used to determine the flight location with respect to the ground, apparently to position the moth at the height where the cladodes will be easily detected. Second, CO$_2$ gradients of just a few ppm on a background of 350 ppm are detected in the vicinity of the cladodes. The ability of *Cactoblastis* to resolve these gradients is so acute that it can distinguish the more robust cladodes that have higher concentrations of photosynthetic enzymes and hence are more nutritious. This allows the insect to select the best locations on which to lay its eggs.

**Olfactory CO$_2$ perception in a high-CO$_2$ world**

In most insect species capable of using CO$_2$ as an olfactory signal, anthropogenic increases in atmospheric CO$_2$ are not likely to have major impacts because the CO$_2$ levels being sensed are higher than what is predicted to occur. The survival of many lepidopteran species, however, may be threatened because their ability to resolve a given CO$_2$ signal is weakened as the background CO$_2$ level increases (Stange, 1997). In addition, the CO$_2$-receptor neurons of highly responsive species such as *Cactoblastis cactorum* are adapted for high sensitivity at low CO$_2$ but approach signal saturation at double current atmospheric CO$_2$ levels (Stange, 1996, 1997). CO$_2$-receptor neurons are temperature compensated, and this capacity is also weakened as the background CO$_2$ level increases (Stange and Wong, 1993). Blood-sucking insects may also be adversely affected, mainly in their ability to resolve small signals in the activation response. As a result, they may be more sluggish in initiating search behavior. However, because host animals produce high CO$_2$ concentrations in the breadth, the ability to find the host once the activation response has been initiated will likely be little affected (Stange, 1996).

**Conclusions—Global Change and the Sensing of CO$_2$**

The CO$_2$ rise of the past century, and what is predicted for the coming century, represent a return to atmospheric CO$_2$ levels not seen for 10 to 20 million years, if not longer. Most of the research associated with rising CO$_2$ has focused on plants because of the central role of photosynthesis. In plants, responses to high CO$_2$ are relatively benign or improve perfo-
formance by improving the carbon and water balance of the plant. By contrast, the ability of certain insects to detect food by variation in atmospheric CO$_2$ is compromised because their ability to resolve small gradients declines as the background level of CO$_2$ increases. With large enough increases in atmospheric CO$_2$, the ability of these creatures to sense CO$_2$ could be reduced enough to threaten their survival, unless they can adapt and recover an ability to detect subtle changes in CO$_2$. It is not known whether this will be possible in an atmosphere with a high background level of CO$_2$. Because a large number of insect species potentially use CO$_2$ as a cue, the consequences of their lost CO$_2$ sensitivity could be great. Even in remote, relatively pristine areas of the globe, substantial stress could be imposed on insect populations that lose their ability to use CO$_2$ to identify food sources. Global insect biodiversity could be further eroded, with unpredictable consequences as the loss of insect populations cascade to other trophic levels.

Ironically, however, it may be the insect sensory system that may provide key insights into how plants sense CO$_2$. In plants, no CO$_2$ receptors have been identified, although they may be central to the ability of the stomata to respond to CO$_2$. An intriguing possibility is that CO$_2$ receptors in insect sensilla may be homologous with plant CO$_2$ receptors, should they exist. Identification of the genes for the insect CO$_2$ receptors could allow for sequence comparisons with homologues from plant genomes, possibly leading to identification of plant CO$_2$ receptors. With the completion of the genome for Drosophila and Arabidopsis, such possibilities may soon become reality.

REFERENCES


