Roles and Effects of Environmental Carbon Dioxide in Insect Life

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Abstract

Carbon dioxide (CO_2) is a ubiquitous sensory cue that plays multiple roles in insect behavior. In recent years understanding of the well-known role of CO_2 in foraging by hematophagous insects (e.g., mosquitoes) has grown, and research on the roles of CO_2 cues in the foraging and oviposition behavior of phytophagous insects and in behavior of social insects has stimulated interest in this area of insect sensory biology. This review considers those advances, as well as some of the mechanistic bases of the modulation of behavior by CO_2 and important progress in our understanding of the detection and CNS processing of CO_2 information in insects. Finally, this review briefly addresses how the ongoing increase in atmospheric CO_2 levels may affect insect life.

INTRODUCTION

Carbon dioxide (CO$_2$) is an important constituent of the chemical environment, and insects possess specialized receptor cells that can detect and measure environmental CO$_2$ sensitively. It is well known that CO$_2$ plays roles in the biology of insects despite being a ubiquitous sensory cue. Recently, those basic roles have been extended to an increasing number of insects, and similarities in its use appear to arise even among insects with rather different habits. After years of speculation, the mechanisms underlying transduction of CO$_2$ stimuli at the level of receptor-cell dendrites are currently being unraveled, and processing of CO$_2$ cues in insect brains also is beginning to be understood. This research is contributing to our understanding of olfactory mechanisms in insects. Also, as many insects use CO$_2$ as a cue to find resources, expanding our understanding of the CO$_2$ sensory system may lead to improved strategies for surveillance and control of harmful insects.

The global atmospheric concentration of CO$_2$ is rising markedly as a result of human activities. The average level has increased from about 280 ppm (µl/l) immediately before the Industrial Revolution to a daily average of 380 ppm in 2005 and is increasing at a rate of about 2 ppm per year (58). Present levels far exceed the natural range over the past 650,000 years (180–300 ppm). Such an increase in CO$_2$ levels is expected to affect the biology of a number of living organisms including insects and therefore is stimulating research on what those effects could be. Moreover, this aspect of global change highlights the importance of understanding the roles of CO$_2$ in the biology of insects at present and past CO$_2$ atmospheric levels. Owing to space constraints, this review emphasizes recent hypotheses and findings on the roles and effects of CO$_2$ in insects.

BEHAVIORAL ROLES OF CO$_2$

Role of CO$_2$ Sensing in Insect-Plant Interactions

In the past decade dramatic progress has been made in our knowledge of the behavioral roles that CO$_2$ plays in the interaction between insects and their host plants. Even when much work remains to be done, there is evidence suggesting multiple roles in this interaction.

Oviposition behavior. Adult Lepidoptera possess an organ in their labial palps, the labial-palp pit organ (LPO), which is specialized for sensing CO$_2$ (17, 63). In the pyralid moth *Cactoblastis cactorum* this organ is vestigial in males and relatively prominent in females, and thus it was suggested that CO$_2$ plays a role in the oviposition behavior of these moths (95, 97). *C. cactorum* is a nocturnal moth that oviposits on *Opuntia stricta*, a Crassulacean acid metabolism (CAM) plant. These plants, found mainly in arid regions, assimilate CO$_2$ during the night and thus are sinks for CO$_2$. Plants that have more photosynthetic activity during the day receive more eggs during the night (79). It is thought that these moths sense the magnitude of the negative CO$_2$ gradients associated with a plant's metabolic activity and thus detect the healthiest plants, that is, the plants that assimilate more CO$_2$ during the night (95). Theoretical estimates suggest that a gradient of CO$_2$ occurring within a boundary layer of about 5 mm over the surface of the plant implies a concentration difference of more than 80 ppm, well above the detection threshold of the CO$_2$-detection system (below 40 ppm) of these moths. Moths probe the surface of a plant with their LPOs before ovipositing, apparently to measure this gradient (97). Therefore, CO$_2$ acts at short range for *C. cactorum*.

A role for CO_2 in oviposition behavior was also proposed for the sphingid moth *Manduca sexta* (1), although in adult *M. sexta* there is no apparent sexual dimorphism in the LPOs (63). A preferred host plant of *M. sexta* is a strong source, not sink, of CO_2 during the night (50), and thus this moth would use the high CO_2 levels generated by the plants as cues to evaluate their physiological status (1). Because *M. sexta* females do not probe the plant surface before ovipositing, CO_2 cues may affect the behavior of these moths at a distance from the plant, but it is not known if CO_2 acts at long or short range or both. The evidence obtained so far for these species is indirect. Experiments involved masking of CO_2 stimuli using methods (e.g., generation of fluctuations of artificially high CO_2 around test plants) that could, for example, affect the plants themselves and therefore alter other olfactory cues. To obtain direct evidence, plants emitting different natural levels of CO_2 should be offered in a choice test. In the diurnal butterfly *Pieris rapae*, there is no clear evidence of a relationship between photosynthetic rates (CO_2 assimilation) in plants and the number of eggs laid on them (72, 73). Moreover, no data are available on the direct effects of CO_2 on the oviposition behavior of this insect.

Direct evidence for a role of CO_2 in the oviposition behavior of herbivorous insects, however, is available (94, 96). For example, tephritid flies, economically important parasites of fruit, oviposit into small existing lesions in the skin of fruits. Such lesions release volatile compounds including respiratory CO_2. CO_2 alone, released at levels similar to those emitted from lesions, attracts gravid flies at short range (centimeters) (96). Responses are dose dependent in the range 100–3000 ppm above ambient CO_2 levels. As CO_2 alone attracts flies at levels lower than that of CO_2 in a natural, complete stimulus (fruit lesion; 96), other volatiles may increase the attraction by CO_2. The effectiveness of such a ubiquitous stimulus may result from context specificity, because in the experiments CO_2 was released from a small source over

and close to a fruit-like substrate, a context that simulates a lesion in a fruit (96).

As a result of insect oviposition, Scots pines (*Pinus sylvestris*) systemically reduce their rate of photosynthesis—that is, their rate of CO_2 assimilation—and thus trees bearing eggs become weaker CO_2 sinks than trees without eggs (87). Therefore, the number of eggs that a plant carries could modulate sensory responses of insects via CO_2 cues.

Foraging behavior. Many coleopteran larvae that feed on living or decaying plant material use CO_2 as a cue to find food (59, 80). Furthermore, CO_2 is the only volatile compound that larvae of the beetle *Diabrotica virgifera virgifera* use when orienting themselves toward corn roots (12). In addition, larvae of the noctuid moth *Helicoverpa armigera* preferred sources of high CO_2 alone in a Y-maze choice experiment in which the control stimulus was CO_2-free air (85). Field observations support a role of CO_2 in their foraging behavior, because those larvae feed on plant tissues that do not assimilate but are sources of CO_2 (mainly fruits and flowers). Behavioral responses of *H. armigera* larvae are dose dependent, reaching a maximum at 800 ppm CO_2 (85). Larvae of the pyralid moth *Elasmopalpus lignosellus* also orientate toward sources emitting a range of CO_2 concentrations in an olfactometer (mainly at 1000–1500 ppm) (55).

Whereas it has been known that CO_2 cues play a role in the foraging behavior of lepidopteran larvae, it was not clear whether adults may also use those cues. Many adult lepidopterans feed on nectar, and it has long been assumed that flowers are important sources of CO_2 (53, 89). Production of nectar and CO_2 in the nocturnal, moth-pollinated flowers of *Datura wrightii* are positively correlated temporally (50), so that floral CO_2 emission may indicate food abundance to the moths. This idea is strengthened by the results of a choice experiment using the moth *M. sexta* (109), whose main host plant in the Sonoran Desert (Arizona) is *D. wrightii*. In a flight cage

a foraging, naïve moth was offered two plants, each bearing a single scented, nectar-free surrogate flower. The control flower emitted CO_2 at approximately ambient levels, and the test flower emitted CO_2 at 750 ppm, approximating the CO_2 emission typical of newly opened, highly profitable *D. wrightii* flowers (50). Most moths directed their first proboscis extension into the flower emitting the higher level of CO_2 (109), and therefore it was suggested that, as in the cases of other (e.g., hematophagous) adult insects, nectar-feeding insects may also use CO_2 as a foraging cue. These experiments did not clarify whether CO_2 serves as a cue for orientation to, or only as a local feeding stimulus at, a flower. The behavioral response of *M. sexta* to CO_2 is plastic because if CO_2 is not associated with a reward, then moths learn not to prefer sources of high CO_2 (109). Plastic responses to CO_2 in the context of foraging are also found in honey bees (71).

The idea that moths use CO_2 as a cue for foraging could imply that they can predict nectar volumes before probing the flowers and would hover in front of (or land on) only promising flowers, thus increasing their foraging efficiency. This hypothesis assumes that in the field, at the time of moth foraging, there is a positive spatial correlation between nectar volumes in and CO_2 emissions by the flowers. This spatial correlation has not yet been demonstrated. Moreover, how a moth's visit (involving partial or full depletion of nectar) might affect such a spatial correlation between nectar and CO_2 emissions remains to be tested rigorously.

In *Drosophila melanogaster*, CO_2 cues have been suggested to play a local role in the selection of profitable food sources (36). In an olfactometer, adults and larvae avoid CO_2 when presented alone at 1000 ppm above ambient levels or at higher concentrations (36, 104). These responses have been correlated with CO_2 release from fruits: Unripe bananas emit more CO_2 than ripe ones, and flies prefer ripe fruits. The repellent effect of CO_2 was suggested to depend on the odor context (36). Elevated CO_2 is also found in an odor blend released by stressed *D. melanogaster* and avoided by responding flies (104). Whether CO_2 can be attractive to the flies in any natural context is not yet clear.

Role of CO_2 Sensing in Insect-Vertebrate Interactions

CO_2 emanating from vertebrates helps blood-sucking (hematophagous) insects detect and orientate toward a host and thus plays a role in foraging (76). Such a role for CO_2 in foraging of mosquitoes was recognized long ago (20, 42, 105, 107). CO_2 alone activates and modulates the host-seeking behavior of mosquitoes in the laboratory and in the field (32, 40–42, 107), albeit less effectively than do complete, natural stimuli (20, 39). The fine-scale structure of the CO_2 plume strongly influences the behavioral responses of mosquitoes in a wind tunnel (29, 40). Whereas activation occurs with either a homogeneous or filamentous plume, upwind flight (also called attraction) and source-finding are observed mainly when the plume is filamentous, i.e., when flying mosquitoes are exposed to intermittent increases in concentration. Such a plume is what mosquitoes would encounter naturally at a relatively long distance from a host, and it was proposed that CO_2 plays an important role as a long-range orientation cue (123). Under filamentous conditions the response threshold falls below 500 ppm over ambient levels, and responses increase with doses up to 40,000 ppm above ambient levels (40). CO_2 also acts at close range, stimulating probing of the host's skin (32, 94). It is thought that CO_2 is an important attractant component for mosquitoes with generalist feeding habits, whereas CO_2 is less important for specialists with a more specific host range and sensitivity to specific skin odors (41, 76, 106, 107).

In attracting mosquitoes, CO_2 acts synergistically with the human skin odorant L(+)-lactic acid (20, 28, 32, 39, 105), which evokes a variable level of response (33, 39). In a Y-tube olfactometer a mixture of CO_2 with

lactic acid attracts more mosquitoes than the arithmetic sum of the attraction to those two stimuli when presented alone. No such synergistic effect, however, is observed for activation (39). CO_2 also acts synergistically with 1-octen-3-ol (41, 105, 107), a vertebrate odorant present in human sweat (22), in attracting mosquitoes. Brief exposure to CO_2 instantaneously sensitizes (i.e., reduces the threshold for) mosquito attraction toward an upwind source of skin odor (27). Such sensitization may help mosquitoes identify and locate a host and may play a role in the synergistic responses described above.

For tsetse flies, CO_2 acts as a behavioral activator and induces attraction and landing (41, 76, 118). In the field, attraction by CO_2 is enhanced synergistically in the presence of the ox-breath odorant acetone (110). In wind-tunnel experiments, a source emitting a turbulent plume of CO_2 alone at a level as low as 50 ppm above ambient attracts the flies (35), which suggests that CO_2 plays a role in long-range attraction. In habitats of tsetse flies, ecologically relevant CO_2 sources can be detected by a CO_2 sensor as far as 60 m downwind (122). At such a distance from the source, a CO_2 plume exhibits intermittent pulses less than 10 ppm above ambient levels and about 100 ms in duration. It could be assumed that an insect's CO_2-sensing system can detect those stimuli (46, 93, 122). Therefore, CO_2 would act as a long-range attractant, although its effect would be enhanced by other vertebrate odorants.

Responses of walking larvae and adults of triatomine (kissing) bugs (Reduviidae) to CO_2 have been tested mainly in locomotion compensators and olfactometers. In contrast with adult mosquitoes and tsetse flies, triatomines showed little (47, 108) or no (6, 81) activation in response to CO_2. Tests in which CO_2 was produced by a culture of yeast, however, suggested an important activating effect (48), although it is unclear if other emitted odorants contributed to this effect. As for other hematophagous insects, the triatomines orient upwind in air streams enriched with CO_2 alone (6, 19, 47, 81, 108) or in combination with synthetic odorants (6, 7, 82). Unlike adult mosquitoes, however, walking kissing bug larvae can orient in homogeneous plumes of CO_2 alone (8). To understand better this interspecific difference, such studies should be extended to other flying and walking hematophagous arthropods. Orientation toward CO_2-enriched airstreams has a threshold of 350 ppm above ambient levels for CO_2 presented alone and 100 ppm above ambient when CO_2 is presented simultaneously with L(+)-lactic acid (6), suggesting synergism between the two stimuli (when presented alone, lactic acid does not attract the bugs). Responses to CO_2 are found only during the first hours of scotophase, when the insects are seeking a host, and this circadian rhythm is controlled endogenously (9). CO_2, however, is not a necessary odor cue for attraction of triatomine bugs to hosts (7, 47, 81, 108). Moreover, at least some mosquitoes can be attracted by host odors lacking CO_2 (11, 18, 41, 91).

A number of other hematophagous arthropods also use CO_2 in host-seeking (21, 25, 41, 84, 86, 101, 111). Notably, and contrary to what has been found for phytophagous insects, CO_2 apparently plays no role in the oviposition behavior of the sheep blow fly (Calliphoridae) (113).

Role of CO_2 Sensing in Insect Social Behavior

Insect social behavior includes the control of environmental variables inside nests. For example, excessively high temperatures or CO_2 levels within nests can have deleterious effects on the colonies. As part of the control of nest-climate conditions, some social insects measure and regulate the levels of CO_2. Regulation in honey bees involves collective ventilation by wing-fanning workers near the hive entrance, which actively drives air out of the hive (88, 92). Thus, under natural conditions, there is a positive temporal correlation between CO_2 levels in the hive and the number of fanning bees (88). Large colonies control

CO$_2$ levels more precisely and at lower levels (mean CO$_2$ level of 4400 ppm) than do small colonies (mean CO$_2$ level of 7800 ppm).

Colonies of bumble bees (*Bombus terrestris*) also control nest climate through fanning activity (116), and the number of fanning bees increases as CO$_2$ levels increase. The response threshold of colonies with up to 120 workers is about 16,000 ppm (115, 116), which roughly agrees with field studies in which that value was never reached. The individual fanning behavior underlying the collective control of CO$_2$ levels is variable (115). Thus, the threshold, probability (at above-threshold stimuli), and duration of fanning responses are independent parameters involving significant interindividual variability. Response thresholds decrease over successive trials, leading to specialization in individuals with a low response threshold (i.e., individuals that respond more frequently) (115). Specialization is believed to be a key factor in colony organization, leading to increased colony efficiency.

In ants the structure of the nest and surface wind are important for ventilation (65) and ensure relatively low interior CO$_2$ levels (66). CO$_2$ values do not exceed 28,000 ppm in giant nests of *Atta vollenweideri*, a figure similar to that reported for termite nests (94). As for bees, CO$_2$ in large nests is maintained at lower levels than in small nests (66). Ant workers can alter the shape of the nest's channel openings. It is thought that this could represent a relatively slow but long-term response of a colony to unfavorably high CO$_2$ levels (65).

DETECTION AND CNS PROCESSING OF CO$_2$ INFORMATION

Studies of the detection and processing of CO$_2$ cues should contribute to our understanding of the molecular and neural bases of CO$_2$-related behaviors. This in turn may help us understand the behavioral roles of CO$_2$ and also may lead to the development of methods for the surveillance and control of harmful insects.

Detection of CO$_2$ Cues by Sensory Cells

The physiology of the CO$_2$ receptor cells (RCs) of different insects has been studied in detail. Those studies suggest that CO$_2$ RCs are not typical odorant receptor cells (ORCs).

Sensory physiology. Highly sensitive CO$_2$ RCs have been found in a number of insect species in mouthparts or antennae, but not in more than one appendage in a single species (98). Typically, CO$_2$ RCs housed in thin-walled sensilla with wall pores have dendrites with an increased distal surface area (67, 90, 98). Those sensilla usually contain a single CO$_2$ RC, although a second CO$_2$ RC (17, 63, 75), ORCs (26, 44, 121), or a thermoreceptor cell (68) also might occur in the same sensillum in different species. In some cases, sensilla sensitive to CO$_2$ are clustered to form a specialized sensory organ. On the basis of morphological findings, it has been proposed that CO$_2$-sensitive structures in insects have a polyphyletic origin (98), and other arthropods (e.g., centipedes, ticks, terrestrial crabs) also possess CO$_2$ RCs (100, 101, 119).

The lepidopteran LPOs, in the distalmost segments of the labial palps, contain a number of sensilla that vary from a few to more than 1000 depending on the species (63, 98). All those sensilla contain, apparently exclusively, RCs specialized to detect CO$_2$ (15, 46). The variability in the numbers of LPO sensilla is not understood, but it may be related to differences in feeding habits (63). For example, moths that do not feed as adults have vestigial LPOs (63). In moths, LPO RCs are selective, are highly sensitive, and can represent CO$_2$ levels over many orders of magnitude (46, 93, 97). In the moth *Heliothis armigera*, the response threshold for the LPO as a whole was estimated to be just 0.5 ppm over ambient levels (93).

The CO$_2$ RCs of moths can encode rapid changes in the level of CO$_2$ through a phasic component of their responses. Those

ORCs: odorant receptor cells

RCs: receptor cells

responses are bidirectional, so that the CO_2 RCs can signal both increases (through excitation) and decreases (through cessation of spiking) in concentration. In both cases responses are proportional to the change in concentration, so that they simultaneously signal both direction and rate of change (46). The phasic component of the responses could follow short, intermittent increases in CO_2 level for stimuli delivered at a frequency as high as 10 Hz, suggesting a role in tracking a plume of CO_2 during long-range orientation (46, 93). In addition, the responses of the CO_2 RCs have a tonic component that does not adapt to prolonged stimulation (except in *C. cactorum*), so that the CO_2 RCs continuously signal the background (environmental) level of CO_2 from CO_2-free air up to levels at least one order of magnitude higher than the current atmospheric concentration (17, 46, 99). Both variable background levels and rapid signal fluctuations are features of natural CO_2 stimuli (46, 50, 94), and the moth's LPO RCs appear to process these features simultaneously.

Among dipterans, the CO_2 RCs of mosquitoes and biting midges are located in basiconic sensilla on the maxillary palps (43, 45, 62), whereas in tsetse flies the CO_2 RCs are located in the antennae (16, 30). As in moths, the physiological properties of the CO_2 RCs of mosquitoes include specificity, sensitivity, likely a broad concentration-response curve, bidirectionality of response, and the ability to signal continuously the CO_2 background levels (44, 45). In mosquitoes, the sensilla that contain the CO_2 RCs also house two other sensory cells, one of which responds to 1-octen-3-ol (45). Because the CO_2 RCs of mosquitoes are in mouthparts and the ORCs tuned to lactic acid are in the antennae, the behavioral synergy between CO_2 and lactic acid originates in the CNS. By contrast, interactions between sensory inputs tuned to CO_2 and 1-octen-3-ol are conceivable. In *D. melanogaster* CO_2 RCs are found in the antennal basiconic sensillum 1 (ab1) (26). This type of sensillum houses four RCs, only one

of which is sensitive to CO_2 (ab1C cell); the other RCs in the sensillum are tuned to different odorants.

In bees and ants CO_2 RCs have been found in sensilla ampullacea on the antenna (68, 98). In ants the sensilla are grouped. Each sensillum consists of an external pore opening into a chamber that connects to the ampulla via a relatively long cuticular duct. As in bees, the ampulla contains one thin-walled wall-pore peg with a single CO_2 RC (67, 98), although a second, thermoreceptive cell is present in a fraction of the sensilla ampullacea of some ants (68). Enclosure of the pegs inside an ampulla somewhat resembles the LPO of lepidopterans. The rationale for these structures is not clear. As in moths and mosquitoes, the CO_2 RCs of ants can signal background levels of CO_2 continuously and without sensory adaptation, which would enable them to assess the absolute CO_2 concentration inside the nest (67).

CO_2 is detected by sensilla that strongly resemble olfactory sensilla, but it has been proposed that the CO_2 RCs function in a qualitatively different way than a typical ORC. Thus, CO_2 RCs function as concentration detectors, which can distinguish between changes in air speed and concentration because adsorption of CO_2 to sensory structures is reversible and there is a continuous equilibrium with the external CO_2. In contrast, typical ORCs function as flux detectors, which do not distinguish changes in speed from changes in concentration and measure the rate of odorant molecules irreversibly adsorbed by the sensillum (61). Additionally, CO_2 RCs bear certain physiological similarities to those responding to changes in ambient temperature and humidity (67, 112, 120). For example, thermoreceptors and hygroreceptors can also respond bidirectionally to step changes in concentration (112). Therefore, the CO_2 RCs could be not only considered ORCs but also included within the group of RCs that monitor environmental variables such as temperature and humidity. Dissimilarities in the structure of the dendrites of typical

Sensory adaptation: decrease in sensitivity of RCs due to the influence of a previous stimulus condition

GR: gustatory receptor

OR: odorant receptor

Antennal lobes (ALs): sites of primary processing of olfactory information in the insect brain

SEG: subesophageal ganglion

Glomeruli: functional modules in the AL, structures of condensed AL neuropil, often spheroidal and surrounded by glia, wherein neurites of AL neurons and terminals of axons of RCs interact synaptically

LPOG: LPO glomerulus

Projection neurons (PNs): AL output neurons that relay synaptically processed information from AL glomeruli to higher centers in the protocerebrum

ORCs and CO_2 RCs, and similarities in those structures between CO_2 RCs and thermoreceptors, have been reported (98). The CO_2 RCs are not typical ORCs.

Signal transduction. The mechanism of transduction of CO_2 stimuli in the dendrites of insect CO_2 RCs has been a matter of speculation. A gustatory receptor (GR) gene (*Gr21a*) is expressed in the CO_2 RCs of *D. melanogaster* (ab1C cells) (104), and the GR gene family is now thought to include genes that encode GRs as well as odorant receptors (ORs). Both GR and OR genes encode seven-transmembrane domain G-protein-coupled receptor proteins. Genetic silencing or ablation of *Gr21a*-expressing cells strongly interferes with a behavioral (avoidance) response of larval (36) and adult (104) fruit flies to CO_2, whereas activation of those cells is sufficient to elicit this behavior (103). Moreover, recently it has been found that *Gr21a* is coexpressed with *Gr63a* in all CO_2 RCs of *D. melanogaster* (60, 70). Coexpression of both GR genes is necessary and sufficient for the CO_2 RCs to respond to CO_2, and it is thought that those GRs form a heterodimeric receptor for the detection of CO_2. Their exact role in CO_2 sensing, however, has yet to be determined.

Orthologs of *Gr21a* and *Gr63a* have been identified in mosquitoes, and they also are coexpressed in the same RCs. Moreover, they are expressed selectively in the maxillary palps, where the CO_2 RCs are located (60). Orthologs of these GR genes also have been identified in a moth and a beetle (70), but not in honey bees, suggesting that their chemoreceptors for CO_2 are different.

Processing of CO_2 Information in the Antennal Lobe

In lepidopterans the axons of the CO_2 RCs project, via the labial-palp nerve, into the CNS at the level of the labial neuromere of the subesophageal ganglion (SEG) and then continue bilaterally, targeting both antennal lobes (ALs) of the deutocerebrum (63, 64, 74). This finding was surprising because it was expected that sensory input from the labial palps would be processed in the labial neuromere of the SEG, not in the primary center for processing olfactory input from the antenna. This unexpected projection pattern suggests that information about CO_2 cues is integrated with sensory inputs from the antennae. Typically, an insect AL comprises an array of condensed neuropil structures called glomeruli, which contain RC axon terminals, neurites of AL neurons, and synapses among those elements. Glomeruli are functional modules of the AL, and CO_2 RCs project into a single, specific glomerulus—in lepidopterans, the LPO glomerulus (LPOG)—in each AL (17, 46, 63, 64, 74). The LPOG receives no primary-afferent input from the antenna and thus appears to be a dedicated target for sensory projections from the CO_2 RCs (17, 63, 64, 74). This finding, together with results of studies of the AL neurons associated with the LPOG, argues that the LPOG is devoted to processing of CO_2 information from the LPO and thus is the CO_2 glomerulus of lepidopterans.

Intracellular recording from and staining of AL neurons have provided physiological and anatomical evidence unequivocally establishing that the AL is the first-order processing center for CO_2 information in the moth's brain (46). The CO_2-responsive projection neurons (PNs) in the AL have dendrites that arborize exclusively in the LPOG. In *M. sexta*, PN axons project into the ipsilateral protocerebrum, mainly to the lateral horn and the calyces of the mushroom body. As found in the case of CO_2 RCs of moths, CO_2-responsive PNs are specific, sensitive, and bidirectionally responsive over a wide range of CO_2 concentrations (46, 49). In addition, those PNs follow short, intermittent stimuli up to a frequency of at least 10 Hz (46), an ability that may enable the moths to follow a plume of CO_2 to locate a CO_2 source from relatively long distances (e.g., meters). It remains to be tested if CO_2-responsive PNs can signal background

levels of CO_2 continuously as do CO_2 RCs of *M. sexta*.

In mosquitoes, the RCs housed in maxillary-palp sensilla have axonal projections into the CNS resembling those of CO_2 RCs of the LPOs of moths. In *Anopheles gambiae* the projections extend bilaterally beyond the SEG and terminate in three glomeruli in each AL that do not receive antennal afferents (4, 5, 57). By contrast, in *Aedes aegypti* the projections are unilateral (ipsilateral) but also terminate in three glomeruli that are not innervated by axons of antennal ORCs (3, 5, 31, 57). The number of glomeruli receiving axonal projections from the maxillary-palp sensilla matches the number of types of RCs in the sensilla that house the CO_2 RCs. This suggests that CO_2 cues may be processed by just one glomerulus in the AL, and that the other two RCs accompanying the CO_2 RCs in the maxillary palp sensilla are olfactory. There is some evidence suggesting that one of the three glomeruli receives input from the CO_2 RCs (10, 57).

In *D. melanogaster*, the CO_2 RCs, located on the antenna, project into a single AL glomerulus. Two-photon imaging with a calcium-sensitive fluorescent protein expressed in all neurons shows that a single AL glomerulus, the V glomerulus, is activated by stimulation with step increases in CO_2 (104). As in the case of the LPOG in moths, the V glomerulus of *D. melanogaster* is a CO_2 glomerulus. Activation of this glomerulus is highly specific and sensitive and has a relatively broad concentration-response curve. Moreover, this activation is necessary and sufficient for a behavioral (avoidance) response (104).

In summary, these recent, first studies suggest that terminals of the CO_2 RCs, located in antennae or mouthparts, project (uni- or bilaterally) into a single glomerulus in each AL. This glomerulus is specialized for processing CO_2 cues, and the PNs arborizing in it exhibit responses to CO_2 that at least partly resemble those of the RCs that provide input to the glomerulus.

PREDICTED EFFECTS OF RISING ATMOSPHERIC CO_2 LEVELS ON INSECTS

The level of CO_2 in the atmosphere has been increasing at an accelerating rate in recent years. Even if human efforts lead to stabilization of that rate in the near future, a value of 570 ppm atmospheric CO_2 is expected for the end of this century, and 770 ppm (double current levels) for 2200 (58).

Indirect Effects on Insects

Increases in atmospheric levels of CO_2 can cause increases in plant growth rates and changes in the physical and chemical defenses of plants and the chemical composition of their tissues (14, 23, 56, 83, 102), and phytophagous insects are indirectly affected by those changes in their host plants. Observed decreases in the protein content and increases of the C/N ratio in leaves under elevated CO_2 (13, 56) imply a reduction in food quality. In some cases herbivorous insects compensate for that change through increased consumption (24, 56, 117). In some communities increased herbivory also may be due to the phagostimulant effects of increased levels of sugar in plants (51). The increase in plant biomass induced by elevated CO_2 may, in turn, compensate for increases in defoliation (24). Some insect populations may not fully compensate for the reduced quality of their diet, while others may overcompensate (13, 37, 56). In addition, in some communities, decreased instead of increased herbivory occurs (69).

Studies of community interactions are useful to evaluate the effects of increased levels of CO_2 in realistic experimental conditions that, for example, include feedback between populations (83). Such studies do not allow discernment between indirect and direct effects on insects, but tests for indirect effects have been carried out. For example, when leaves of two different species of plants are offered to caterpillars in dual-choice experiments, the insects' host plant preference shifts whether the plants

were grown at elevated CO_2 (560 ppm) or at ambient CO_2 levels (2). Thus, the insects' choice is affected by the induced changes in the host plants. Experiments that help to predict the effects of future climate changes on insect populations are, by necessity, becoming more complex. The anthropogenic increase of atmospheric CO_2 levels "very likely" is responsible for the ongoing global warming, as well as for changes in environmental variables other than temperature (58). Therefore, in addition to its own effects, elevated CO_2 could affect insects through changes in those other environmental variables. It has been suggested that the effects of elevated CO_2 should be studied simultaneously with those and other environmental variables of climate change (e.g., with increased temperature and levels of O_3), as the effects of altered climatic variables can interact (37, 38, 54, 56, 83, 117). In addition, impacts of elevated CO_2 on insects through effects on their natural enemies (parasites, predators, pathogens) also are under consideration (24, 54, 77). Moreover, long-term field experiments (preferably involving multiple generations) should provide more realistic data on the variables studied in both plants and insects (56, 114, 117). Because the responses of individual species can be highly variable, it has been suggested that each plant-insect system has some particular responses to elevated CO_2 levels (52, 56). Thus, results from complex studies emphasize the difficulties in generalizing the effects of CO_2 enrichment on ecosystems and the need for more research (38, 56, 78). In any case, a serious impact on insect populations can already be anticipated (24, 34).

Direct Effects on Insects

Very few studies have dealt with direct effects of high atmospheric levels of CO_2 on insects. Knowledge of those direct effects should help to illuminate the mechanistic bases of the effects of high CO_2 on interactions of insects with plants and enemies and should improve our ability to predict shifts in insect population dynamics and community interactions in future environments.

CO_2 appears to be used by moths as a cue to locate suitable oviposition substrates. Elevated ambient CO_2 levels (720 ppm) may interfere with the oviposition behavior of the moth *C. cactorum*; under these conditions females oviposit fewer eggs (95). The oviposition behavior of the moth *M. sexta*, by contrast, is not affected by elevated CO_2 levels up to at least 1200 ppm (1). The physiological bases of decreased oviposition in *C. cactorum* appear to include sensory adaptation of CO_2 RCs when continuously exposed to CO_2 levels of 600 ppm or greater. Under these conditions the CO_2 RCs cannot encode CO_2 information, suggesting that these CO_2 RCs are genetically adapted for detection of CO_2 fluctuations around atmospheric levels that prevailed before the Industrial Revolution (97). In the moth *M. sexta* and other insects, the CO_2 RCs do not adapt to such levels of CO_2. Testing of more insect species is needed to determine if *C. cactorum* is an exception. Insects that exploit hosts that are CO_2 sinks (e.g., *C. cactorum*) may be more affected by the global increases in atmospheric CO_2 levels than insects that use hosts that are CO_2 sources, because the latter evolved in a niche where they frequently encounter relatively high levels of CO_2 (1).

In the moth *H. armigera*, whose CO_2 RCs do not adapt to high levels of CO_2, other effects are seen. After exposure to CO_2 levels above 400 ppm, those CO_2 RCs respond to changes in temperature, in addition to changes in the level of CO_2 (99). This finding suggests that under these conditions the CO_2 sensory system confuses fluctuations in CO_2 with fluctuations in temperature, which presumably could affect behaviors in which CO_2 plays a role. At about pre-Industrial Revolution CO_2 levels, the CO_2 RCs do not respond to temperature changes, supporting the idea that they are genetically adapted to past atmospheric levels of CO_2. These studies show that

increased levels of atmospheric CO_2, such as those predicted for the near future, can affect the physiology of the insect's CO_2-sensing system per se.

SUMMARY POINTS

1. CO_2 may play a role in the foraging behavior of nectar-feeding insects.

2. While host-seeking, mosquitoes find a source of CO_2 at a distance only if they encounter a filamentous plume of CO_2 (that is, when they are exposed to intermittent increases in CO_2 concentration), whereas homogeneous plumes do not lead to source-finding. This contrasts with the behavior of walking kissing bugs, which can orient themselves using homogeneous plumes of CO_2 while seeking a vertebrate host.

3. In host-seeking mosquitoes, brief exposure to an increase in CO_2 concentration instantaneously sensitizes their attraction toward an upwind source of skin odor.

4. As previously thought, CO_2 RCs are not typical ORCs.

5. The CO_2 RCs of *D. melanogaster* coexpress two GR genes, which are necessary and sufficient for the response of CO_2 RCs to CO_2.

6. CO_2 RCs, located in either the antenna or the mouthparts, project their axons into a single glomerulus in the AL, which is specialized for processing information about CO_2 stimuli. This suggests that information about CO_2 cues is integrated centrally with olfactory information detected by the antennae. However, behavioral responses to CO_2 alone (i.e., without integration of odorant information from the antenna) occur.

7. Despite difficulties in generalizing some of the effects that the ongoing increase in atmospheric CO_2 levels will have on ecosystems, a serious impact on insect populations can be predicted.

8. Increased levels of atmospheric CO_2, such as those predicted for the near future, can affect the physiology of the insect's CO_2-sensing system.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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