CO₂ adds another channel—and then some—to plant-pollinator interactions

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In this article I comment on our findings that floral carbon dioxide (CO₂) can be used by Manduca sexta hawkmoths in a scale- and context-dependent fashion. We firstly found, in wind tunnel assays, that diffusing floral CO₂ is used as long-distance cue (e.g., meters). Moths track CO₂ plumes up-wind in the same manner they track floral odors. Nevertheless, CO₂ did not appear to function as a local stimulus for flower probing, evidencing a scale-dependent role in nectar foraging. These results were further enriched by a second finding. In dual choice assays, where moths were offered two scented artificial flowers of which only one emitted above-ambient CO₂-levels, female Manduca sexta chose to feed on the CO₂ emitting flower only when host-plant volatiles were added to the background. We discuss this apparent measurement of oviposition obligations during foraging in the context of the life histories of both insect and plant species. These findings seem to pinpoint the usually artificial nature of compartmentalizing herbivory and pollination as different, isolated aspects of insect-plant interactions. Insects do not seem to have a defined response to a certain stimulus; instead, motor programs appear to be in response to composite arrangements of external stimuli and inner states. If animal-plant interactions have evolved under these premises, I believe it may prove beneficial to include a non-linear, integrative view of plant multi-signaling and life history aspects into the study of pollination biology.

Pollination Biology: Plant Signals and Animal Responses

Since Kölreuter and Sprengel, the study of the behavioral responses of animal pollinators to plant signaling has become a central theme in pollination biology. The diversity and abundance of the class Insecta have made them principal actors amongst the cast of animal pollinators. Plants advertise their rewards with a wealth of floral cues, while nectivorous insects utilize their sensory and cognitive abilities to exploit such information while foraging. Most angiosperms use odors, color displays, alluring shapes and probably other, unknown floral stimuli, to attract insects to their nectar reward (reviewed in ref. 7).

In our studies, we focus on the foraging behavior of the nocturnal hawkmoth Manduca sexta (hereafter: Manduca). In recent years Raguso and collaborators have experimentally manipulated floral signals to both dissect and reconstruct the constellation of stimuli used by Manduca during nectar foraging. The results of these studies have lent support to the idea that pollinators make use of multiple sensory channels whose central integration in the brain has profound consequences for the insect’s behavior. However, despite the insights that we have gained through the use of artificial flowers on the relative importance of olfaction, vision, mechanoreception and hygoreception in nectar foraging by Manduca, we remained skeptical. To what extent did our artificial flowers realistically offer the full complement of stimuli presented by natural flowers? Obviously, to address this question we needed to turn to the natural nectar sources of Manduca. In the Sonoran desert of Arizona, the night-blooming Datura wrightii (hereafter: Datura) is a preferred nectar source for Manduca moths. Datura is also a preferred host-plant for Manduca larvae, which adds an unusual twist to the putatively mutualistic relationship between Datura flowers and adult Manduca pollinators, and made it worthwhile to study in the context of our interests.

The Breath of Datura: A Matter of Scale

The candle-like flower buds of Datura undergo impressive elongation, nectar secretion and scent biosynthesis prior to opening, suggesting high metabolic expenditures associated with flowering. Datura flowers open abruptly, releasing clouds of carbon dioxide (CO₂) at concentrations up to 200 ppm above the ambient air. Our colleagues at the University of Arizona (Guerenstein et al.) had shown that CO₂ levels at the corolla opening are initially correlated with floral nectar production, suggesting that the interests of foraging moths would be served by an ability to detect floral CO₂. Indeed, Manduca is capable of detecting minute (0.5 ppm) differences in CO₂ concentrations with its CO₂-sensing labial-pit organ LPO. and Thom et al. showed that naïve male Manduca first probe on artificial flowers emitting levels of CO₂ observed in freshly opened Datura flowers. These observations convinced us that CO₂ was a candidate for a novel floral component that Manduca could use in its foraging bouts. But, at what scale might it function? Does CO₂ elicit probing responses in the fashion that appetitively conditioned odors trigger Manduca’s proboscis extension reflex PER? Does CO₂ also mediate orientation behavior towards individual flowers? Continuing with our experimental strategy of dissecting and reconstructing floral...
stimuli to better understand insect foraging behavior, we tackled these questions.

We have recently shown, using an experimental wind tunnel, that Manduca adults can utilize CO$_2$ while nectar foraging from artificial flowers emitting CO$_2$-rich plumes at concentrations produced by Datura flowers.

In a series of experiments we found CO$_2$ to act only at a distance: moths track CO$_2$ up-wind in the same fashion they track floral odors, but CO$_2$ shows no effect on the flower probing response. Once a moth reaches the flower’s threshold, fragrance seems to be a reliable local indicator of a feeding site. The effect of CO$_2$ proved to be scale-dependent; from a distance (3 m) it acted as an attractant, or orientation cue, but within mere cm of the flower it seemed to have no effect as a local feeding stimulus. However, if moths can track floral odors from both a distance and close range to evaluate nectar sources, why would they need to track CO$_2$? A possible answer to that question might be rooted in the moths’ olfactory physiology. Because CO$_2$-receptor cells can respond to both increments (excitation) and decrements (inhibition) of the CO$_2$ concentration, they may detect fluctuations with higher temporal resolution than the olfactory receptor cells and neural circuitry that process floral odors, which typically respond to increments in concentration. Both at the periphery (LPO) and at the central nervous system level, fluctuations in [CO$_2$] can be monitored at a temporal resolution of at least 10 Hz, whereas fluctuations in typical floral odors (e.g., linalool) are usually monitored at lower frequencies. This could allow moths to more efficiently search for high-CO$_2$ emitting flowers from a distance, potentially facilitating more accurate predictions of flower profitability “on the wing”.

The Breath of Datura: A Matter of Context

A second experiment, involving dual-choice assays, suggested additional reasons why floral CO$_2$ might be important to the Datura-Manduca relationship. When given the choice between a scented paper flower and an identical second flower with above-ambient levels of CO$_2$, we discovered an unexpected sexually dimorphic behavior. Even though both sexes responded similarly to the floral stimuli in the first experiment, in the dual choice assays of the second experiment, males unequivocally probed the CO$_2$-emitting flower first, while female first choices were random. This result was puzzling, and suggested that foraging and oviposition tasks might be conflated. However, previous investigations had not explicitly addressed this possibility. Experiments conducted to evaluate the effect of CO$_2$ on female Manduca oviposition behavior showed that moths did not have any strong preference between Datura plants surrounded by above-ambient vs. near-to-ambient [CO$_2$]. However, in these experiments the Datura plants did not have flowers.

It has long been known that Datura is both a nectar source and a host plant for Manduca, whose active periods for foraging and oviposition overlap considerably. Despite this pattern, historical research on these two tasks in Manduca—and on pollination and herbivory in general—has tended to separate them intentionally. Thus, we omitted host plant odors in our previous experiments with floral stimuli. Thom et al. used only males to study CO$_2$ effects on foraging behavior and Abrell et al. used plants with no flowers to study oviposition. However, female _M. sexta_ often mix feeding and ovipositing bouts and their reproductive fitness benefits directly from a 10-fold increase in mature egg production when they feed as adults. With this in mind we designed a third experiment, in which we presented moths with the exact same dual-choice as before, but now with host-plant (tomato) odors in the background. This time, males and females responded identically, with unambiguous preference for the flower with high [CO$_2$]. Now, in addition to the scale-dependence of CO$_2$ found in our first experiment, we identified a context-dependent role for CO$_2$. Host-plant odors provided the context in which females chose flowers with high CO$_2$. How could we explain these results?

**Looking into Herbivory to Understand Pollination?**

It has recently been shown that female Manduca oviposit more eggs on Datura plants whose floral nectar volume has been experimentally increased. In that study, the authors suggest that nectar volume could measure into the oviposition behavior of female Manduca, probably as a way to “evaluate” host-plant physiological state. Thus, nectar volume would be a local proxy for host-plant quality. In other words, by orienting towards a plume of CO$_2$, Manduca _sexta_ females might increase the probability of finding nectar-filled flowers belonging to a suitable host-plant. Subsequent successful feeding would then, at the local scale, lower thresholds for oviposition.

**Herbivory Versus Pollination: Why Different Parcels?**

Our study adds carbon dioxide to the list of plant primary metabolites having a “secondary function”, but also depicts an interesting, contextual function of respiratory CO$_2$ in the relationship between _Manduca sexta_ and _Datura wrightii_. In the first place, our findings compel us to consider that plant signals of diverse physical natures, acquired through different sensory channels, are integrated as an assemblage of stimuli, where scale and context acquire crucial relevance. In second place, our study suggests it is a mistake to consider a priori that pollination and herbivory are separate, isolated categories of plant-animal interactions. Perhaps this assumption reflects that a large proportion of our knowledge on pollination biology comes from studies on social insects with division of labor i.e., honeybees, bumblebees, wasps (see ref. 31). Lepidopterans and numerous other solitary pollinators often perform tasks such as foraging, mating and oviposition on limited spatial and temporal scales. The context-dependence of “foraging” responses by Manduca females to floral CO$_2$ suggests that this plant-insect interaction has been molded by selective pressures extending beyond the strict imperatives of foraging and cross-pollination for insect and plant, respectively. We suspect that this phenomenon might be more common than is currently appreciated (see ref. 28), especially considering the relative neglect with which solitary pollinators have been studied. Comprehensive, integrated studies that consider life histories and their vicissitudes may yield more profound insights in the study of insect-plant interactions, as revealed by the breath of the sacred Datura.

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**Herbivory Versus Pollination:**

| Herbivory | Pollination |
|-----------|------------|
| Feed on plant tissue | Feed on flowers |
| Reproductive fitness | Reproductive fitness |
| Habitat destruction | Pollination service |

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