

# How useful are olfactometer experiments in chemical ecology research?

Daniel J. Ballhorn<sup>1,\*</sup> and Stefanie Kautz<sup>2</sup>

<sup>1</sup>Department of Biology; Portland State University; Portland, OR USA; <sup>2</sup>Department of Zoology; Field Museum of Natural History; Chicago, IL USA

**O**lfactometer experiments, in which arthropods are given the choice between two or more odor sources to test behavioral preferences, are commonly used in chemical ecology research. Results of such often lead to conclusions on behavior in an ecologically relevant setting. However, it is widely unknown how well these experiments reflect actual behavior in nature. Recently, we used natural insect herbivores of wild lima bean plants to evaluate their behavior in Y-tube olfactometer experiments compared with feeding experiments. We demonstrated that depending on volatile concentration, insect sex significantly determined preference, and that independent of sex, the actual feeding choice of insects depended on defensive short-distance cues, which did not correlate with volatile cues emitted by the plants. Thus, our study shows that olfactory decisions do not reflect actual feeding choice and that olfactometer experiments may only provide a limited and simplified picture of actual decision making by insects.

Olfactometer experiments are commonly used in chemical ecology research to study how arthropods locate their hosts.<sup>1-3</sup> In particular, the host searching behavior of parasitoid wasps, which lay their eggs inside insect herbivores, has been studied in great detail.<sup>4</sup> It has been shown that such wasps use herbivore-induced volatile organic compounds (HI-VOCs) emitted by plants under herbivore attack to localize hosts for oviposition.<sup>5</sup> Thus, HI-VOCs lower herbivore pressure on the plant and function as an indirect defense mechanism. In contrast to plant-parasitoid signaling, the effects of herbivore induced volatiles on herbivores of the same or

different species are much less studied.<sup>6,7</sup> Beyond this, virtually no quantitative data is available on how long-distance signaling via HI-VOC corresponds to “on-site” plant cues that actually determine feeding behavior of herbivores.

To contribute filling this lack of understanding, in our recent study, we tested to what extent volatile organic compounds emitted at different concentrations are used by herbivores (thus plant antagonists themselves) to localize their food plant. Further, we evaluated how the actual feeding choice of beetle herbivores corresponds to findings from olfactometer experiments. We found that herbivores do indeed use HI-VOCs as orientation cues, but that chemical short-distance cues of the plants overruled the effect of long-distance cues. Specifically, we could show that: (1) HI-VOCs released from wild lima bean plants had very different effects on chrysomelid herbivores depending on the degree of induction, that is the amount of HI-VOCs released, and the sex of the beetle; and that, (2) chemical defensive leaf traits (cyanogenic glucosides) overruled the long-distance signaling provided by volatiles.<sup>8</sup> These results reveal that using olfactometer trials alone without experiments on additional factors driving insect choice behavior, such as quantitative variation of the emitted signal, sex of the insects tested and feeding behavior on the actual plants, are not sufficient to draw profound conclusions. Thus, it can be inferred that olfactometer experiments used to analyze the attractiveness of host plants only provide limited information regarding the actual behavior of insects on the plant.

Plant volatiles emitted into the air are similar to an open book, which makes them

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\*Correspondence to: Daniel J. Ballhorn; Email: [ballhorn@pdx.edu](mailto:ballhorn@pdx.edu)

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widely available to numerous organisms from different trophic levels, including other plants, herbivores and carnivores.<sup>9-11</sup> As such freely exposed information, HI-VOCs often provide very unspecific cues. To overcome this problem, as an example, many parasitic wasps are capable of olfactory learning, which enables them to link host presence to specific odors and thereby increase the chances to distinguish reliable plant signals from unreliable ones.<sup>4,12</sup> Therefore, wasps are able to read the information that is essential to them. For herbivorous insects in particular, information regarding the quality of the plant as a food source or oviposition site is critical.<sup>13-15</sup> In addition to the presence of competitors or enemies, the quality of a plant is determined by the amount and combination of nutritive compounds, tissue water content, or toughness, and non-volatile defensive compounds.<sup>16,17</sup>

In our field study, we used wild lima bean as the experimental plant. Lima bean is characterized by cyanogenesis, the release of toxic hydrogen cyanide in response to cell damage, such as caused by herbivory.<sup>18</sup> Lima bean is an obligate cyanogenic plant with different genotypes showing variation in the concentration of cyanide-containing precursors, however, no acyanogenic lima bean plants are known.<sup>19,20</sup> The amount of cyanogenic precursors is a crucial factor determining attractiveness as food to both generalist and specialist herbivores.<sup>21,22</sup> Generally, plant tissues that are lower in cyanogenic precursor concentration are consumed more in no-choice experiments. In choice experiments, insects prefer low- over high-cyanogenic tissues.<sup>18,23</sup> So then, the question arises to what extent do HI-VOCs convey reliable information on the suitability of individual lima bean plants, and plants in general, as a food source? Does the information carried by HI-VOCs indicate the quantity of defensive compounds in the leaf? In previous research, we showed a quantitative trade-off between HI-VOCs and the concentration of cyanogenic precursors.<sup>19</sup> Plants capable of emitting high amounts of HI-VOCs are low cyanogenic and vice versa. Thus, low cyanogenic plants emitting high amounts of HI-VOCs should be the most attractive to chewing herbivores.

Accordingly, in order to identify the least toxic plants, in our present study, herbivores should have selected the plants that emitted the highest amounts of volatiles in olfactometer experiments. However, this was not the case across our entire study.

On the contrary, beetles avoided plants that emitted the highest amounts of volatiles. This was true for both chrysomelid species used (*Gynandrobrotica guerreroensis* and *Cerotoma ruficornis*). In consecutive feeding trials, leaf consumption was significantly negatively correlated with leaf cyanogenic content, which is in accordance to numerous other studies.<sup>24-26</sup> Thus, the attraction or deterrence of beetles in the Y-olfactometer did not correspond to the concentration of leaf toxins—even though these critically determine food plant quality. In terms of indicating chemical food plant quality, olfactometer experiments can provide misleading information, and results obtained in such experiments might not be of high ecological relevance. However, high concentration of HI-VOCs may also indicate high competition which also detrimentally affects the overall quality of a plant as a potential host.<sup>27</sup> Whether or not herbivore colonization rates or the degree of damage and the subsequent quantitative variation in the release of HI-VOCs by wild lima bean plants in the field corresponds to long-distance attraction or deterrence remains to be tested.

Using olfactometer experiments, we could show that volatile concentration is an additional critical factor that affected behavioral responses in insect beetles. We used four different concentrations of jasmonic acid (JA: 1.0 mmol; 0.1 mmol; 0.01 mmol; 0.001 mmol), as well as herbivore damage, to create different induction levels and compared the attractiveness of these induced plants to non-induced controls (treated with water instead of JA). However, only when considering insect sex in addition to induction level, we were able to fully interpret the observed choice behavior in the olfactometer. Female beetles were equally repelled by all induction levels and always preferred non-induced controls over induced plants. Males, however, were only repelled by the highest induction level (1.0 mmol JA), responded equivocally to plants induced with 0.1

and 0.01 mmol and 0.01 mmol JA while preferring 0.001 mmol JA and herbivore induced plants over controls. This shows a very differential behavioral response by male beetles and again can be explained by the fact that males are often in search of female mating partners.<sup>28</sup> Consequently, slightly induced plants might indicate the presence of females to males making such plant material highly attractive in long-distance behavioral trials. Females, in contrast, are often in search of suitable oviposition places and need to carefully select suitable hosts for the next generation and avoid competition.<sup>15</sup> This finding from our study shows the need to discriminate between sexes in behavioral experiments with insects as generalizations can lead to wrong conclusions.

## Conclusion

In our study, neither insect sex nor induction level of plants played a role in feeding trials. As discussed above, concentration of cyanogenic precursors was the only detected factor that significantly affected feeding choice and overruled long-distance decision making. However, there are multiple factors we did not consider in our study and which potentially determine herbivore behavior, as well. For example, we conducted all our experiments from August to October, after the rainy season had started, and did not test for responses in other seasons. Season, however, might be another aspect critically affecting insect decisions. Males might only respond positively to induced plant material during the mating season and females might only be extremely sensitive to induced plants when they are ready to deposit eggs. At the same time, insect age, which we did not consider in our study, might affect decision making. Older insects might be more experienced and respond more carefully. This factor, possibly among others, should also be considered in future behavioral studies. In summary, olfactometer experiments can provide useful information on long-distance orientation by insects, but differential analyses are required in order to draw ecologically meaningful conclusions. Our study shows that the exclusive use of olfactometer experiments to analyze insect behavior is questionable.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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