

## Article Addendum

# Olfactory recognition of host plants in the absence of host-specific volatile compounds

Host location in the black bean aphid, *Aphis fabae*

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The black bean aphid, *Aphis fabae*, responds behaviorally to the odor of its host plant faba bean (*Vicia faba*) in olfactometer bioassays by spending more time in the treated than control regions. We have shown previously that a blend of fifteen volatile compounds emitted by *V. faba* elicits the same response as a headspace sample of an intact *V. faba* plant. Here we report that no single compound within this blend fully accounts for the behavioral response and that the responses to individual compounds are different when in the context of the blend. As none of the compounds are specific to the host, we have hypothesized that *A. fabae* responds preferentially to the blend of compounds when presented in a species-specific combination of volatiles or in ratios specific to *V. faba*. Future plans to test which of these two hypotheses pertains to host-seeking *Aphis fabae* are discussed.

## Host Recognition in the Black Bean Aphid, *Aphis fabae*

The black bean aphid, *Aphis fabae* (Hemiptera: Aphididae), is a serious pest of faba bean, *Vicia faba* (Fabales:Fabaceae), causing damage both directly by feeding and also by acting as a vector for plant viruses.<sup>1-4</sup> *Aphis fabae* over-winters on its winter host, spindle (*Euonymus europaeus*) before migrating to a wide variety of summer hosts including *V. faba*.<sup>5</sup> Wingless forms develop and reproduce on the summer host with crowded conditions leading to the production of winged summer forms which fly to other plants, leading to a rapid spread throughout crops. In an attempt to identify novel means of controlling this pest, the use of olfaction in winged summer morphs to locate new hosts has been investigated. A greater understanding of

this mechanism could lead to ways of disrupting the ability of aphids to locate new hosts and so limit colonization of crops.

We have shown previously that *A. fabae* responds behaviorally to a 15-component blend of electrophysiologically active volatile compounds emitted by *V. faba*.<sup>6</sup> This blend consists of (*Z*)-3-hexen-1-ol, 1-hexanol, (*E*)-2-hexenal, benzaldehyde, 6-methyl-5-hepten-2-one, octanal, (*Z*)-3-hexen-1-yl acetate, (*R*)-(-)-linalool, methyl salicylate, decanal, undecanal, (*E*)- $\beta$ -caryophyllene, (*E*)- $\beta$ -farnesene, (*S*)-(-)-germacrene D and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) at the same concentrations and ratios as identified in the headspace of an intact *V. faba* plant. As none of these compounds are specific to *V. faba*,<sup>7-11</sup> we hypothesized that *A. fabae* uses a host-specific blend rather than a single volatile compound to locate its host.

## Behavioral Responses of *Aphis fabae* to Individual Volatile Compounds of *Vicia faba*

A four-arm olfactometer was used to determine the behavioral responses of winged summer migrant *A. fabae* to each of the compounds in the original 15-component blend using the same method as described in Webster et al.<sup>6</sup> Only one compound, (*Z*)-3-hexen-1-ol at a dose of 85.8 ng, elicited a similar behavioral response to the 15-component synthetic blend, with the aphids spending significantly more time in the treated region of the olfactometer than the clean-air controls ( $p = 0.047$ ) (Fig. 1). However, when aphids were offered the choice between (*Z*)-3-hexen-1-ol and the synthetic blend in an olfactometer choice test, the aphids preferred the synthetic blend over (*Z*)-3-hexen-1-ol alone ( $p = 0.017$ ) (Fig. 2). This indicates that (*Z*)-3-hexen-1-ol does not fully account for the response to the synthetic blend and so host location in *A. fabae* is not mediated by a single compound alone.

Interestingly, for three of the compounds; octanal, (*R*)-(-)-linalool and (*S*)-(-)-germacrene D, the aphids spent less time in the treated region of the olfactometer compared to the controls. We expected that removing these three compounds from the blend would result in the aphids responding more strongly, preferring the reduced component blend to the original. However, when an olfactometer choice test was carried out, the aphids showed no preference for the reduced blend over the original ( $p = 0.976$ ) (Fig. 3). This highlights

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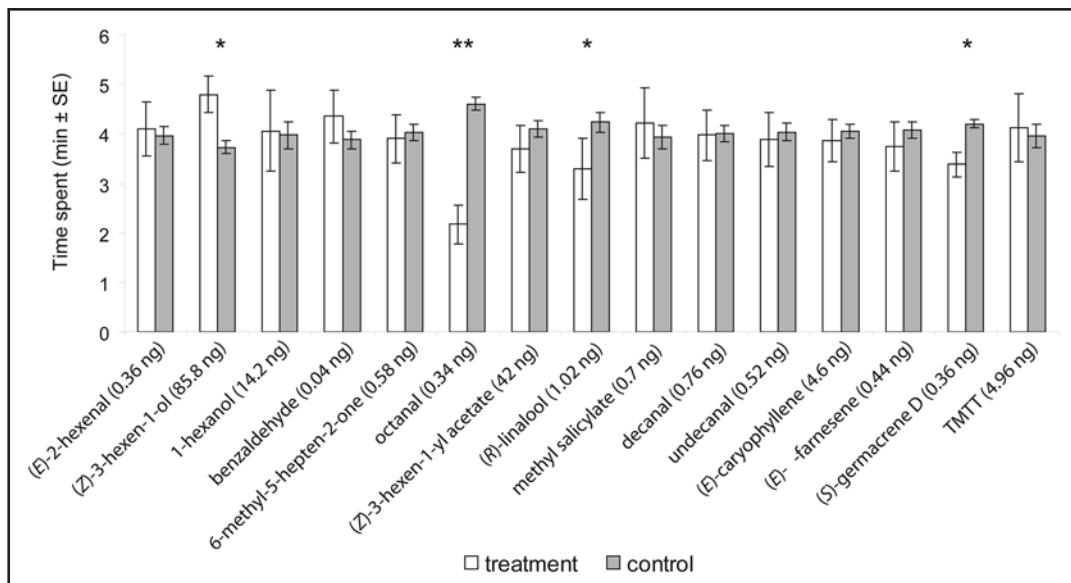


Figure 1. Behavioral response of winged virginoparae *Aphis fabae* to individual compounds at same concentration as determined in air entrainment sample. \* Significantly different ( $p < 0.05$ ) \*\* Significantly different ( $p < 0.01$ ).

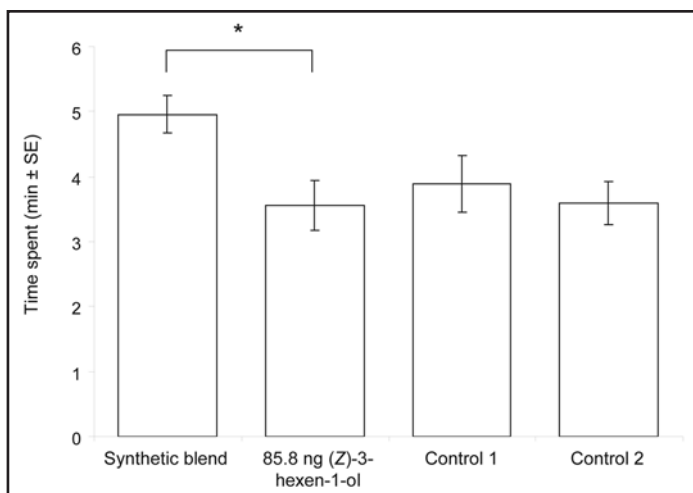


Figure 2. Behavioral response of winged virginoparae *Aphis fabae* to synthetic blend and 85.8 ng (Z)-3-hexen-1-ol when both odor sources present together in the same olfactometer. \* Significantly different ( $p < 0.05$ ).

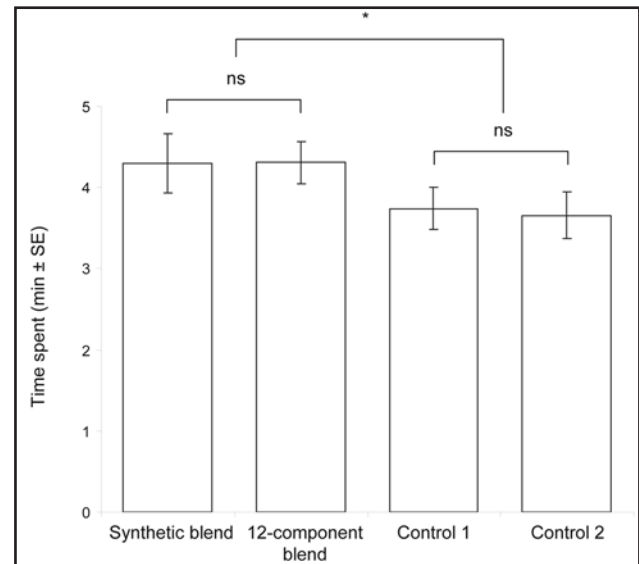


Figure 3. Behavioral response of winged virginoparae *Aphis fabae* to synthetic blend and twelve-component blend when both odor sources present together in the same olfactometer. \* Significantly different ( $p < 0.05$ ).

the behavioral importance of the blend over individual compounds. When presented alone, these three compounds caused the aphids to spend less time in the treated region of the olfactometer but, when in the context of the blend, they had no detectable effect on the aphids' behavior.

### Host Recognition Using a Blend of Ubiquitous Plant Volatile Compounds

There are two possible hypotheses that could explain how a blend of ubiquitous plant volatiles could be used by a host-seeking insect to recognize its host:

- (1) Recognition of a species-specific combination of volatiles
- (2) Recognition of a blend of volatiles in a species-specific ratio

Hypothesis 1 depends on a response by the insect to a host-specific combination of volatile compounds. Although each of the volatile compounds in the synthetic blend is found in a wide range of unrelated plant species, it may be that *V. faba* is the only species to emit a certain combination of them. To test this hypothesis, it would need to be demonstrated that non-host plants do not emit the same combination of volatile compounds. This would be a difficult task as the number of non-host plants that would need to be analyzed is immense. Useful information could still, however, be obtained from analyzing a small number of non-host plants.

Hypothesis 2 depends on a response by the insect to species-specific ratios of volatile compounds.<sup>12</sup> Testing this hypothesis would

first mean entraining a large number of *V. faba* plants to determine if the ratios of key volatile compounds remained consistent from plant to plant and then determining if the range of ratios does not overlap with those of non-host plants. The former task would be relatively simple. Previous work involved the entrainment of a large number of potato plants and quantification of key volatiles produced by each plant. This showed that the ratios of volatiles emitted remain fairly consistent from plant-to-plant.<sup>13</sup> A similar approach could be taken to determine if the same is true for volatiles of *V. faba* and future experiments are planned to answer this question.

For both hypotheses, then, the question of how insects can use blends of ubiquitous volatile compounds to avoid non-hosts remains a difficult question to answer due to the large number of non-host plants. Valuable information could still be obtained by looking at a smaller number of non-host plants but criteria for selecting which non-host plants to analyze are required.

*Aphis fabae* exists as a number of host races; each race specialized on a number of summer host plant species. Previous work has shown that specialization on a particular host can result in fitness trade-offs, restricting the ability to develop on other hosts.<sup>14</sup> Behavioral preference for the optimal host would provide a selective advantage to *A. fabae* and this has been found to be the case for some host races.<sup>15</sup> Studies have also shown that different host races can adapt to developing on novel hosts over time and modify their preferences accordingly.<sup>15,16</sup> Determining whether or not preference for these different hosts is mediated by species-specific blends or ratios of ubiquitous volatiles would provide an excellent starting point in determining whether or not aphids employ either of these methods to locate suitable hosts and avoid those which are less suitable, allowing the investigation of these two hypotheses with a manageable number of plant species.

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