

Involvement of odorant cues in the process of superparasitism avoidance

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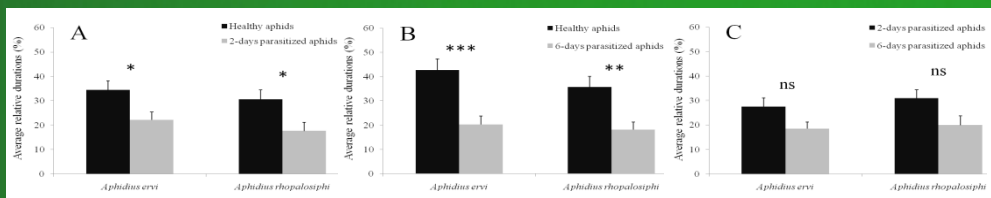
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Abstract

The ability to avoid superparasitism provides a selective advantage to parasitoid females, allowing them to avoid depositing eggs in lower quality host. We observed in a Y-olfactometer that generalist aphid parasitoids, *Aphidius ervi* and *Aphidius rhopalosiphii* (Hymenoptera, Braconidae), were more attracted toward non-parasitized than parasitized *Sitobion avenae* (Hemiptera, Aphididae) colonies. We collected the odors released from healthy aphids and aphids parasitized for 2 and 6 days using an electronic nose. *Sitobion avenae* alarm pheromone, (E)- β -farnesene (EBF), was the only chemical identified, and was found in lower quantities in parasitized aphids. Both parasitoid species provided pronounced electrical depolarizations to EBF in electroantennography (EAG), and both were attracted to the latter compound in the Y-olfactometer. Parasitoid attraction was known to be guided by a variety of odorant cues released by plants and hosts, and our results support the hypothesis that the aphid alarm pheromone acts as a kairomone for *A. ervi* and *A. rhopalosiphii*.

1. Y-Olfactometer: Discrimination between healthy and parasitized aphids

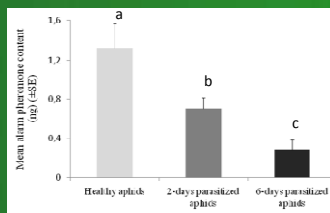
Y-olfactometer assays demonstrated that volatile chemicals are likely to be involved in the discrimination process, as both parasitoid species spent significantly more time in the olfactometer arm leading air from non-parasitized aphids, whatever the time that separated the parasitism and the experiment (2 or 6 days).



However, parasitoid females could not differentiate aphids that have been parasitized for 2 or 6 days.

These results indicate that a modification in volatile emission occurred between the first and the second day of parasitism.

2. Volatile quantification: Semiochemicals released by healthy and parasitized aphids



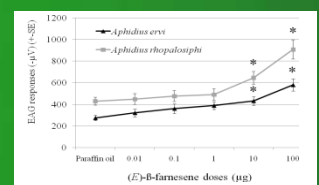
Using an electronic nose (zNose[®]), we found a polynomial relationship between the amount of EBF placed in the vial (X) and the SAW detector response (Y) as: $Y = 5639.3X^2 + 3601.5X + 1559.6$ ($R^2 > 0.99$). The average amount of EBF produced by a single aphid was 1.28 ± 0.26 , 0.70 ± 0.11 and 0.28 ± 0.10 ng (mean \pm SE) for healthy, 2-days and 6-days parasitized aphids respectively (n=12).

Due to the non equality of variances, we analyzed our results through a Welch test (Welch, 1951). This one-way analysis of means (not assuming equal variances) showed that the amount of alarm pheromone produced varies according to the aphid parasitism state ($F_{2,20.414} = 8.0702$, $P = 0.003$).

3. Electroantennography: Perception of (E)- β -farnesene

We observed a positive dose-response relationship in EAG to EBF, both with *A. ervi* ($F_{5,114} = 6.95$, $P < 0.001$, $n = 20$) and *A. rhopalosiphii* ($F_{5,114} = 9.98$, $P < 0.001$, $n = 20$) antennae. Dunnett's post-hoc test showed in both species that the two highest EBF doses elicited electrical responses significantly higher than the paraffin oil control.

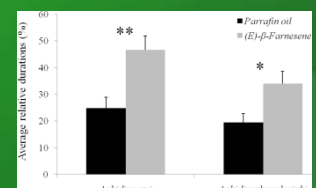
Our electroantennography study revealed that, as many aphid predators including ladybeetles and hoverflies, the two *Aphidius* species tested in this study have an olfactory system adapted to the perception of EBF.



4. Y-Olfactometer: Attraction to (E)- β -farnesene

In the Y-olfactometer, both *A. ervi* and *A. rhopalosiphii* females were attracted toward the aphid alarm pheromone source (tobs=3.32; $P = 0.001$ and tobs=2.55; $P = 0.012$, respectively).

The branch of the olfactometer conducting the EBF source was also the first choice of *A. ervi* females ($\chi^2 = 6.81$, $P = 0.009$), while *A. rhopalosiphii* did not choose one branch more often than the other as a first choice ($\chi^2 = 3.38$, $P = 0.066$).



Conclusion

According to the results presented in this study, we suggest that EBF acts as a kairomone by parasitic wasps. Already parasitized aphid colonies release less EBF than healthy ones, resulting in a reduced attraction of natural enemies.