



REPLY TO NEGRI ET AL.:

Air pollution and health impacts on bees: Signs of causation

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In their Letter, Negri et al. (1) point out four concerns with our recent publication (2). The first is that the study is correlative, and the next two that we did not measure the toxicity of air pollution directly, nor how the particulate matter (PM) measured on the surface of the cuticle and internal organs could be impacting health. Finally, the authors are concerned with the identity of the elements we found using scanning electron microscopy coupled with X-ray spectroscopy (SEM-EDX).

First, some specific responses. The assertion that we did not investigate the contact between PM and the internal organs is erroneous and is already shown in figure 2 (crop and midgut of ref. 2). As to the identification of the elements with EDX, we did observe elements other than those listed, but we only presented a subset, chiefly metals, in our table as noted in figure 2 of ref. 2, and these elements were identified from several samples. As to their rarity, we also note in *SI Appendix, Materials and Methods* of ref. 2, that we could not quantify their abundance with our methods. However, rare earth (3) and radioactive (4, 5) elements have been previously found in atmospheric PM and aerosols.

Regarding the title of their Letter, we already acknowledged in the original article (2) that the connection between air pollution and the impacts we found on our wild migrating bees is by nature correlative as we could not control for age, diet, source colony, or physiological condition. Thus, we exposed laboratory-reared, caged, fed, and age-matched fruit flies to the same field conditions over several short 10-d periods

to examine whether we found similar effects, which we did. Furthermore, the specific sites were chosen to control for other obvious potential factors such as pesticide use (see *SI Appendix*, table S1 of ref. 2). Finally, we assessed these impacts over a 3-y period where, of the parameters measured, only PM differed across the sites. It is furthermore unlikely that the air pollution composition itself was consistent over this time period (6, 7). These multiple lines of evidence suggest that air pollution is the most likely factor contributing to the impacts we observed.

We agree that “studies correlating insect health with airborne PM levels are important to understand the effect of pollutants in real-world conditions.” The main objective of our study was to determine whether air pollution could be impacting wild systems under field conditions in currently the most polluted country in the world. As acknowledged both in the original article (2) and the Letter (1), air pollution is a complex mixture of airborne toxins and further studies are needed to understand the specific components and concentrations at which our observed effects are found. Raising awareness and discourse on this issue for India and the world was our primary goal, which, as evidenced by this Letter, we accomplished. Finally, we also agree that our study “leaves several unanswered questions,” and we encourage the authors and other scientists across the world to continue to address this important topic to better inform international air quality guidelines. If all we get is cleaner air out of the process, we would be happy with that, too.

1. Negri, G. Capitani, M. Pellicchia, Airborne particulate matter and health effects on bees: A correlation does not indicate causation. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 26576–26577 (2020).
2. G. G. Thimmegowda et al., A field-based quantitative analysis of sublethal effects of air pollution on pollinators. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 20653–20661 (2020).

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Author contributions: G.G.T., A.B., P.S.D., and S.B.O. wrote the paper.

The authors declare no competing interest.

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First published October 13, 2020.

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