

Interactive comment on “Spores of most common airborne fungi reveal no ice nucleation activity” by B. G. Pummer et al.

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Referee#3: If they were contained in the oil phase, this would be an alternative reason why they did not show any ice nucleation activity in the experiments. Since the fungal spores are rather large, it should be possible to spot them in a light microscope with large magnification. The authors should therefore add a figure to the manuscript with microscope images that show the location of the fungal spores within the emulsion.

Hydrophobic IN may partition to the oil phase instead of the water phase.

How many evaluated droplets were in the field of view of the microscope?

Answer: The method has proven valid for different types of IN, like pollen, mineral dusts and Snomax at different concentrations (Pummer et al. 2012 and unpublished data).

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We added a figure (now the new Fig. 2) to show fungal spores in the emulsion. As it can be seen, fungal spores distribute into both phases, so that every droplet contained several spores. Even though fungal IN molecules can separate from the spore itself (see Kieft and Ruscetti 1990, Pouleur et al. 1992) and might then move into the oil phase, this separation is not complete, therefore at least some activity should remain in the aqueous phase.

We additionally did some reference measurements with a few selected species in the setup by Fröhlich-Nowoisky et al., and added a corresponding chapter in the paper.

Referee#3 The experiment has to be discussed in more detail to exclude a misinterpretation of the absent IN activity.

In addition, whether droplets contain IN or not depends on the droplet size distribution and the IN size distribution. Therefore additional information should be provided for the emulsion preparation and data evaluation procedure covering the following questions: For how long were the samples emulsified with what stirring speed (RPM)? How stable were the emulsions?

What was the size distribution of the droplets?

Were only the larger droplets considered for evaluation (as stated in Pummer et al., 2012)?

How many times was a freezing experiment repeated?

How was the reproducibility?

What was the spread of freezing temperatures? E.g. add freezing temperature for 1 or 5 % activated fraction to table 2.

Answer: We added more information about the handling of the setup (see p.5/L.19 – p.6/L.11) and some statistical information about the measurements (see Table 2): We now show the number of counted droplets and the values for T10 (the threshold where

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10% of droplets are frozen), which is, when compared with the corresponding T50, also a measure for the spreading of freezing events across the temperature axis. We did not determine the initial freezing temperature, since it is statistically less reliable than the median freezing temperature. Furthermore, in any setup it can never be fully excluded that the first droplet freezing is contaminated with a single more efficient ice nucleus.

We mixed our samples by manually shaking them, therefore no stirring speed is available. We always measured our samples right after preparation in order to avoid a potential impact of time, since we wanted to keep the number of free parameters low. From our experiences we know that the sample has to be shaken up again due to sedimentation of droplets and particles in the oil, however, overall it seems to be quite stable.

REFERENCES:

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Pouleur, S., Richard, C., Martin, J. G., and Antoun, H.: Ice nucleation activity in *Fusarium acuminatum* and *Fusarium avenaceum*, *Appl. Environ. Microbiol.*, 58, 2960-2964, 1992.

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