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Interactive comment on “Urediospores of *Puccinia* spp. and other rusts are warm-temperature ice nucleators and harbor ice nucleation active bacteria” by C. E. Morris et al.

Anonymous Referee #1

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Manuscript Title: Urediospores of *Puccinia* spp. and other rusts are warm temperature ice nucleators and harbor ice nucleation active bacteria
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General comments: The topic is very interesting and explores the ice nucleation activity of biological particles: rusts urediospores and their possible impact on climate. Furthermore, an attempt was made to estimate and distinguish the effect of total bacteria and *P. syringae* carried by spores on its ice nucleation activity. This paper addresses an important topic which needs extensive research work. This paper could be improved by focusing on its finding and by discussing its relevance to the issues that are based

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on concrete evidence. In the text, some of the sentences are too long, and wordy. For example line 3-6 in abstract, line 1-5 in introduction. Some part of the result section is not written in a clear, and concise manner. The authors should clearly provide information about the number of experiments have been performed for each test and provide complete statistical analysis to support the conclusion. Every figure should have an appropriate title. The legends should explain clearly the data presented in the table or figures. At the very least, in the result section, the explanation should be clear enough to easily track the sample with its corresponding bar in the graph.

Abstract: Samples collected from Turkey and Syria, and even from Brazil were only used in the preliminary experiment in Table 1 and were not used in the subsequent experiments. Thus, the sampling site should be removed from the abstract. Line 21-26 are speculation and should not be included in abstract.

Introduction: - Line 4-5 give the reference. - Line 25, 26: “Apart from our report “. If you are referring to “our report” why are references from other studies given. - More information should be given to “rust” in the introduction - There has been no concrete mention of the specific goal of this study in the introduction. Furthermore if the conclusion of this study is given on page 5: line 11-14, it is not clear and well written.

Materials and Methods: - Give the abbreviation of KB right after the first time mentioned: “King’s medium B” on page 6: line 3, and not on line 5

Results: 3.1 Table 1: The data obtained from samples collected from Syria (green houses) and Turkey was not used in the subsequent experiments (ex: for their total bacteria, *P. syringae*, and onset of ice nucleation) due to insufficient concentration of spores. They were not included in table 2. The same thing is true for *Coffea arabica* Coffee collected from Brazil (field sample). Thus, it is not even necessary to include them in Table 1. This should have been kept as a preliminary result and not presented for publication. Although, the authors are attempting to present the data for samples

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collected from both field and green houses, in reality there was only one sample (*T. aestivum* cv. Michigan amber, wheat) from green house that showed the onset of INA initially at -9°C and later after two weeks at -6°C . Having only one sample from a green house is not sufficient to be compared with field samples. There should be more samples collected from green houses. In conclusion, all the data was exclusively collected from France with the exception of one from USA (*T. aestivum* cv. McNeal, wheat). Thus, the collection site from Turkey, Syria and Brazil should be removed from line 7-8 in abstract.

Figure 1: - Legend - Please be clear about the strain *P. syringae* CC0670. Was it collected from: i) cantaloupe blight, ii) wild plant of the *Primula* species or both?

- Only very few points are given for the cumulative freezing spectra of rusts. This is not acceptable. Especially for the wild oats and field wheat which have only two points. No reliable conclusion can be made based on this figure. This figure should be redone using more points.

- The full name should be given for rusts species.

3.2 Figure 2: - The word “rate” is not appropriate for this graph. Perhaps a better title would be something similar to: Determination of the concentration ratio for attached total bacteria or *P. syringae* to washed rust spore.

- According to Table 2, there are five (5) plant species that showed the onset of ice nucleation activity (INA) at -6°C ; however, in Figure 2, there are 6 plant species. Please verify this.

- Moreover, in Table 2 there are only two (2) plants with INA at -6°C which showed the absence of *P. syringae*. However in Figure 2. There are three (3) plants showing no data for *P. syringae*.

- Statistical analysis should be done.

3.3

This section is not clear at all. It is based on Table 2 which itself has many column with titles that are confusing and are not described well in Materials and Methods. This section is numerously referred to the content of Table 2; however, it is not specifically evident which column, and how they come up with their conclusion.

Table 2: - Please explain: i) ice nuclei/*P. syringae* cell and ii) ice nuclei/mL due to *P. syringae*

Figure 3: - It does not have any title, and the legend should be rewritten.

There is no statistical evaluation done to analyze the significant importance of one to the other; however, just by looking at Figure 3: - 8 out of 13 samples had very much the same abundance of ice nuclei for both spore and total bacteria (all the samples frozen at -6°C , two of the samples frozen at -8°C , and at -9°C . - 2 out of 13 samples had much lower abundance of ice nuclei per total bacteria, and 3 out of 13 samples had much lower abundance of ice nuclei per spore as compared to both total bacteria and *P. syringae*. - With the exception of one, higher abundance of ice nuclei per *P. syringae* was observed for all the samples.

Thus, except for *P. syringae*, in the majority of the samples, the abundance of ice nuclei was the same for both total bacteria and spore. 3.4

Figure 4: - What was the base for the selection of samples treated by lysozyme in Figure 4? For example, *C. Arabica* was not analyzed for its bacterial content and thus, was not included in Table 2 and in the other figures, yet it was treated with heat and lysozyme. On the other hand, *Avena fatua*, being one of the two samples with the highest total bacteria and ice nucleation activity on set at -4°C , was eliminated as well as *Dactylus glomerata*.

- In spite of having the name: *C. album* in the figure, no data is provided for it.

- The name of the samples affected by lysozyme should be written in the result sections. It is very hard to follow from what is written on page 10: line 2- 5.

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- How many times was this experiment done?

Discussion: The antibacterial property of lysozyme was used to eliminate the role of bacteria in ice nucleation activity in spore. The fact that the author acknowledged in page 11: line: 14-17, that lysozyme can also “react with the oligosaccharides from the hydrolysis of chitin, and chitin is the principal cell wall constituent of the Eumycota”; line: 25-27, “It is plausible that variations in amounts and configurations of chitin oligosaccharides occur during these processes, and that these oligosaccharides were substrates for the lysozyme used in our treatments.” the question remains as why they used this chemical. Furthermore, no explanation was given for the odd observation obtained for four of the samples showing a very high percentage of increase in ice nuclei concentration after the treatment. Depending on the number of times this experiment was done, this observation might be related to just experimental error. Most part of the discussion is mainly based on speculation (ex: pg 13: from line 10, and pg 14) and disperse from their own finding. In the last paragraph, the authors stretch their limited results and connect *Puccinia graminis* f. sp. *tritici* to the possible “impact on cloud processes involving warm-temperature glaciation?” which should be avoided.

Conclusion: The content of lines 23-33 in page 15 is irrelevant with experimental results presented in this paper.

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