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Manuscript Title: Fusarium head blight in the Russian Far East: 140 years of the 'drunken bread' problem

The main objective of this study was to obtain information on the species of fungi associated with cereal grains under favorable conditions in the Amur region of Russian Far East with a focus on Fusarium species, their DNA content/ rate of infection on the grains and their mycotoxin producing potential and identifying the different chemotypes.

Overall, the paper is good and has good information and highlights the problem of *Fusarium* species in cereal grains even after 140 years of work in this field. However, at some places, it was hard to understand what authors were trying to say and there were other incidences where they were verbose. I think the results section needs more references to the tables and figures.

Here are some of my comments/suggestions:

## There is interchanged use of the term 'strains' and 'isolates', please pick one and be consistent throughout the manuscript.

Line 39: High genetic similarity was found among the analysed F. culmorum strains from remote regions was found;

Line 43: grain harvested

Line 44: as a high

Line 55: later Russian migrants were associated with the poisoning of people and animals

Line 66: ...sent the affected plant material to famous Russian 67 mycologists, <u>I do not think this</u> needs to be here.

Line 67: and deposited <u>diseased grain head</u> specimens in herbaria (<u>kept in the Herbarium LEP of our laboratory</u>, <u>the first specimens are dated 1912</u>). Thanks to this inquisitive individual, drawings of typical symptoms of the disease and pathogens were published (Fig. 1). <del>and diseased grain 69 heads are kept in the Herbarium LEP of our laboratory (the first specimens are dated 1912)</del>.

Line 79: The development of multilocus sequence typing (MLST) has facilitated the <u>identification of species and chemotypes</u> <u>assays</u> of the F. graminearum species (Fg) group (Ward et al., 2008). Is this accurate? The sentence did not make sense.

Line 82: According to previous studies bBased on MLST assays, several species of the Fg group, including F. graminearum sensu...

Line 87: parenthesis and year missing in the citation

Line 89-93: A single sentence is a paragraph here. The sentence needs rewording and maybe break into two, it is hard to follow through.

Line 96: The Russian Far East is a region that is often exposed to weather disasters, such as floods, which can lead to negative consequences insurmountable by human efforts and technologieseal methods and cause resulting in significant agricultural damage.

Line 103: Epidemics of FHB in the region fuelled our interest in investigating this disease in the Russian Far East using available modern methods of research.

Line 112: <u>In the mid-August, 2019, The samples of spring wheat (nine samples) and barley</u> (four samples) were harvested <u>from thein different locations of the Amur region, in the Russian Far East in the middle of August 2019</u>. The collection of specimens at <u>these locations fields</u> was approved by the Russian Science Foundation (project 115 number: 19-76-30005).

Line 116: The climatic conditions of 2019 were characterized by excessive moisture: This part of the sentence needs rewording

Line 230: infection by Fusarium spp. fungi; please use Fusarium spp. instead of Fusarium fungi

Line 233-234: *F. sporotrichoides* info is present only in the supplemental data, need to indicate that or say data not presented.

Line 234-238: Data not presented?? Or supplemental table??

Line 237: as well as species fungi of the Fusarium fujikuroi

Line 239: Alternaria spp. were the second frequent genera isolated from the grains. Is this accurate for this sentence? Please avoid the indirect language.

Line 240: Moreover,- the infection of wheat grain with <u>Alternaria spp. these fungi</u>-was almost two times lower

Line 241-243: Data not presented?? Or supplemental table??

Line 263-264: Is this sentence accurate? Please check. Based on the table in supplemental, the ppb values are slightly different and comes from barley samples?

I suggest including the summary tables in supplemental 2 (both mycologia and DNA and MT tabs) as supplementary and reference to it because in the results section there is a good amount of reference to the data in those tables.

Line 272-274: Did not find a reference to TeA and TEN so may be add data not shown in parenthesis for these.

Line 288-290: Among the analysed strains of F. graminearum s. str., nine strains were the 3-AcDON chemotype while 21 strains turned out to be the 15-AcDON chemotype (Table 2).

Line 293: with high bootstrap support (ML/MP/BP: 99/99/1.0). Our phylogentic analysis indicate that strain MFG 60604 is *F. vorosii* and was determined as a 15-AcDON chemotype.

which allows for accurately establishing its 294 species affiliation. This one detected F. vorosii strain was determined as a 15-AcDON 295 chemotype.

Line 296- 298: Three other doubtful strains, MFG 58836, MFG 59052, and MFG 60755, formed the clade 297 with the reference strains F. culmorum NRRL 25475 with high bootstrap support (ML/MP: 298 98/100). All three F. culmorum strains were the 3-AcDON chemotype (Table 2).

I believe the tables should be cited according to their occurrence in the manuscript so please make sure that Table 2 is mentioned before Table 3 in the manuscript or change the table numbers as they appear in the text.

Line 303: and chemotypes prevailed on grain under the most favourable conditions of extremely high...

Line 312-315: Need clarity here, hard to understand what authors are trying to say.

Line 316: 1985–1991, according to published information, the maximal content of DON in grain reached

Line 317: Recently, in 2017, a DON amount of 7,920 ppb was detected in wheat 318 grain grown in southern Europe in 2017 (Kononenko et al., 2020).

Line 320-321: In the plant, ...cereal grains (). This sentence needs rewording.

Line 324: Why is DON-3gl twice as high in barley than in wheat? Could you discuss.

Line 329: understanding that F. graminearum s. str. is the main\_dominant pathogen damaging grain,

Line 330-334: Please rewrite the sentence for clarity.

Line 341-342: needs clarity on what authors are trying to say.

Line 344-346: Again, why is so? Could you discuss that more.

Line 348-351: What could be the reason in the shift? Do 15-AcDON have more fitness advantage???

Line 354: In <u>our analysis addition</u>, <u>we included in the analysis the</u> Fusarium sp. strain MFG 60604 <u>was included</u> that was isolated from wheat grain in the Western Siberia region (the Altai Krai); phenotypically, this strain was a dubious representative of the Fg group. <u>InFor</u> this region,....

Line 358-362: The strain MFG 60604, isolated from wheat grain from West Siberia, was clustered with the reference strain F. vorosii NRRL 45790 with high bootstrap support (ML/MP/BP: 98/99/1.0), which allows for accurate establishment of its species affiliation. This is the third F. vorosii strain found in the territory of Russia and the first one identified in the Siberian region. This is exact repetition of the results needs rewording. May be:

A single strain (MFG 60604) identified as *F. vorosii* in this study, is the only third strain of *F. vorosii* found in Russia and the first one identified in the Siberian region.

Line 363-365: Rewording: Previously identified strains of *F. vorosii* from the Russian Far East belonged to 15-AcDON chemotype and so did the strain identified in this study.

Line 367-369: Needs rewording for clarity. Is this a speculation or is there any evidence, if there is evidence of F. vorosii 3-AcDON chemotype, please cite.

Line 370: In addition, two strains of

Line 372: analysed F. culmorum strains collected from remote regions and characterized by different climatic conditions

Line 380-381: Needs clarity. Is it a single isolate or a single spore isolates, language is confusing.

Line 282-283: Needs rewording for clarity.

Line 384: Did you expect high diversity of *Fusarium* spp or high frequency of *F. asiaticum*? Not clear

Line 393: Fusarium fungi spp.

Line 395: Fusarium fungi spp.

Line 405: The detected maximum <u>DON</u> content of deoxynivalenol in wheat grains reached 13,141 ppb in this study. Multilocus sequence analysis revealed that majority of the isolates used in this study belongeding to F. graminearum s. str.

Fig1: The perithecia, spores of fungi and symptoms of Fusarium disease of cereals from the Far East of Russia, which were drawing and published published in the book by N. A. Palchevsky (1891).

## If possible, need to increase the resolution of this figure.

Figure 3: A little more description of the tree and its content. For eg: Isolates in bold with NRRL number with Fusarium species name are references. What does 92/88/1.0 mean? And maybe the full name of the four loci used with their short forms in parenthesis. F. pseudograminearum was used as an outgroup.

So that the figure could be understood stand-alone. Same for table 3, better description and footnotes.