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VOLATILE ORGANIC COMPOUNDS - CHEMICAL SIGNALS TO COMMUNICATE PLANT HEALTH

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Introduction

Acute outbreaks of plant pathogens and pests are often managed by broad applications of pesticides at the same concentrations across the field, ignoring the uneven distribution of disease at the start of an epidemic. Site-specific management of pests requires reliable and remote detection of the target pests. Visual signals of target diseases on the upper canopy can be captured and interpreted, but receiving these signals from below the upper canopy remains challenging. Volatile organic compounds (VOCs) are chemical signals that are emitted by plants and constitutively or in response to biotic and abiotic stress conditions. These signals can be detected remotely and serve as bioindicators for plant diseases. Rapid development in sensor technology could enable us to exploit these signals for disease detection in the field.

We have determined species-specific VOC profiles of plants infected with Septoria nodorum blotch (SNB) in wheat under greenhouse conditions in a previous study (Ficke et al., 2022). Infection with SNB was associated with emission of 2-Heptadecanone and Mellein 7, 14 and 21 dpi. We hypothesize that **VOCs collected from greenhouse plants can be also found in the field and could act as reliable biomarkers for disease detection**.

Preventing pests from establishing in new geographical regions is the most effective measure to manage plant health. In the new EU funded project PurPest, we are developing VOC-based sensor prototypes to detect five target pest, including insects, nematodes and pathogens and prevent their entry and establishment into the EU (www.purpest.eu, see also yellow box below).

Materials and Methods

Field experiments: In 2021 and 2023 spring wheat 'Bjarne' was sown in the field at the NIBIO station in Ås, Norway. At BBCH 60-70, two groups of 4-5 healthy plants were selected. One group was inoculated with *P. nodorum* conidia suspended in Tween water (0,1%) at 10^5 spores/ml, while another group was mock-inoculated. Both plant groups were covered separately with airtight glass domes (Fig. 1). VOCs were collected over 24 hours directly post inoculation, 7 and 14 dpi over by pushing charcoal filtered air across the plant surface in the glass dome at 240ml/min and pulling the air out at 210-220 ml/min through the lower air inlet using an electric pump. A SuperQ filter was placed in the upper air outlet and changed every 3 hours. VOCs were eluted from the filter using 0,3ml of hexene.

Data collection and analysis: Eluted VOCs were analyzed on the GC-MS and compounds identified using the deconvolution reporting software (DRS, Agilent Technologies A.02.00) combined with the Automated Mass Spectral Deconvolution and Identification System (AMDIS) database as described in Ficke et al., 2022.

Disease severity of SNB was assessed at each collection time point. Relevant VOCs were selected based on their consistent presence in relation to absence or presence of the disease. CART® classification and PCA in Minitab 20 determined the relevance of the selected compounds for placing the samples in the two different groups; 'healthy' or 'SNB' (Fig. 2).

Figure 1. VOC collection in the field: Charcoal filtered air flows over two groups of 4-5 wheat plants and is collected on SuperQ filters for 24 hr.

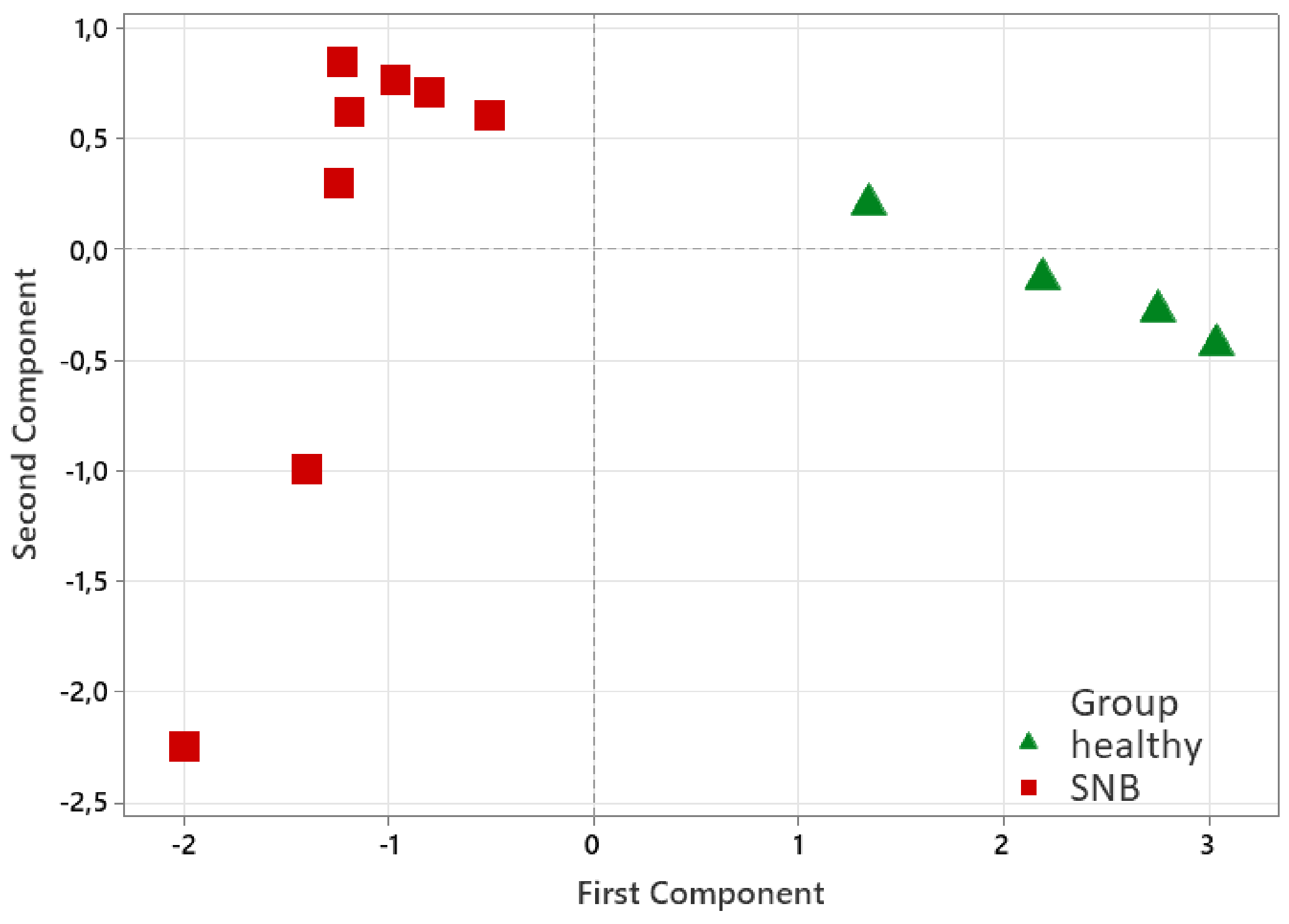


Figure 2. PCA Score plot including 4-Methylacetophenone, α-Cedrene, Z-3-Hexenylacetate, (E)-b-Ocimene and Mellein .

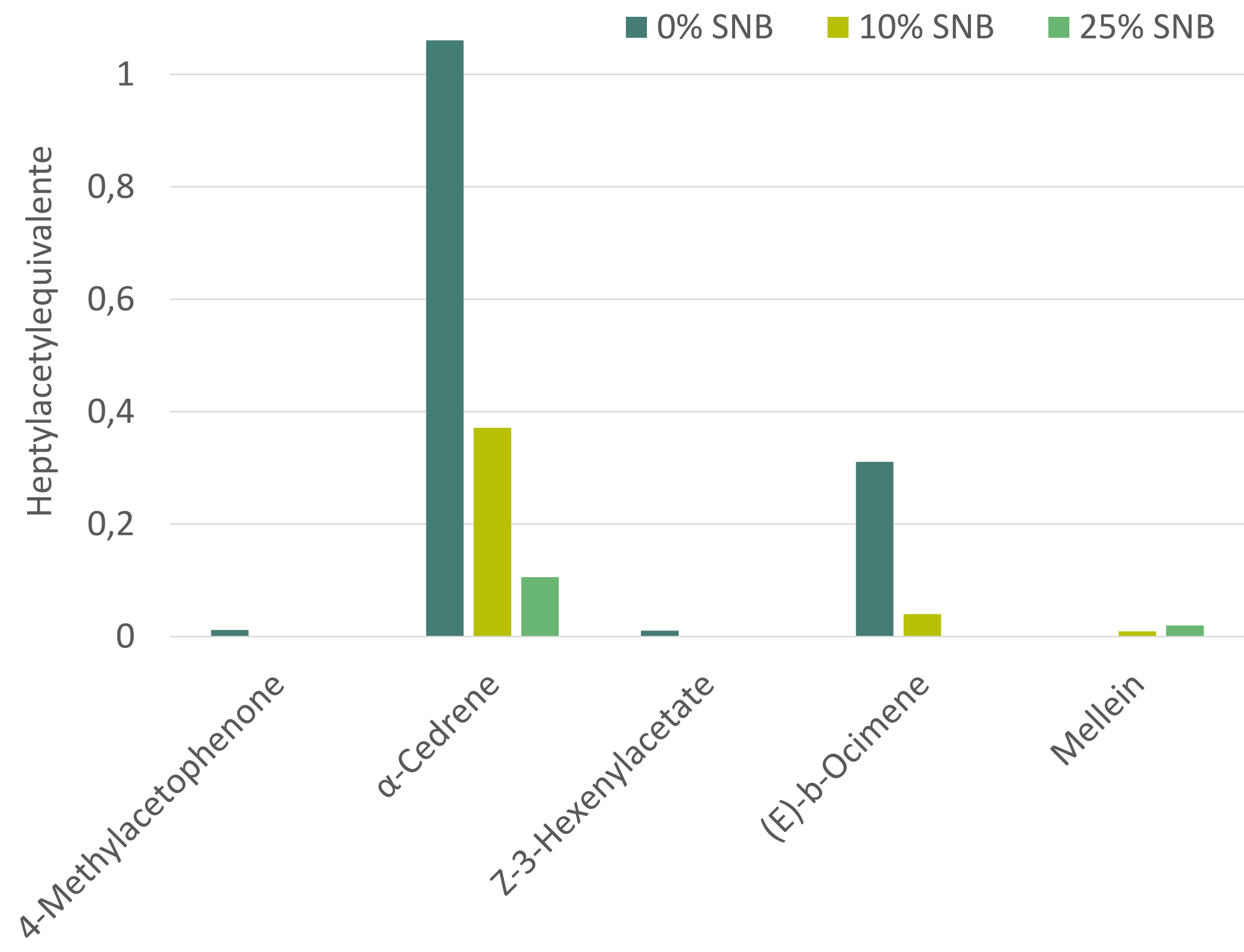


Figure 3. Bargraph showing relative amounts of different VOCs in relation to SNB severity, released wheat plants infected with 0, 10 or 25% SNB.

Results

The average SNB severity of the inoculated group of plants was 0, 5, and 25% in 2021 and 0, 10, and 25% in 2023 after 1, 7, and 14 dpi, respectively. Natural infection of SNB led to 0, 1 and 10% in 2021 and 0, 10 and 25% after 1, 7 and 14 dpi, respectively, on the mock-inoculated plant groups.

The same 33 VOCs were found to be emitted from SNB inoculated and/or mock-inoculated plant groups in 2021 and 2023. Of these 33 VOCs, we selected 5 that appeared to be consistently associated with SNB infection or absence of disease. PCA and CART® classification confirmed that both 4-Methylacetophenone, Z-3-Hexenylacetate and α-Cedrene were important (100%) for classification into healthy and SNB groups, while (E)-b-Ocimene and Mellein were also relevant for placing the samples into 'healthy' and 'SNB' groups (Fig. 2). Compounds associated with the absence of SNB were 4-Methylacetophenone and Z-3-Hexenylacetate, while relative amounts of α-Cedrene and (E)-b-Ocimene declined with SNB infection. Mellein was emitted only when SNB severity was high after 14 dpi (Fig. 3).

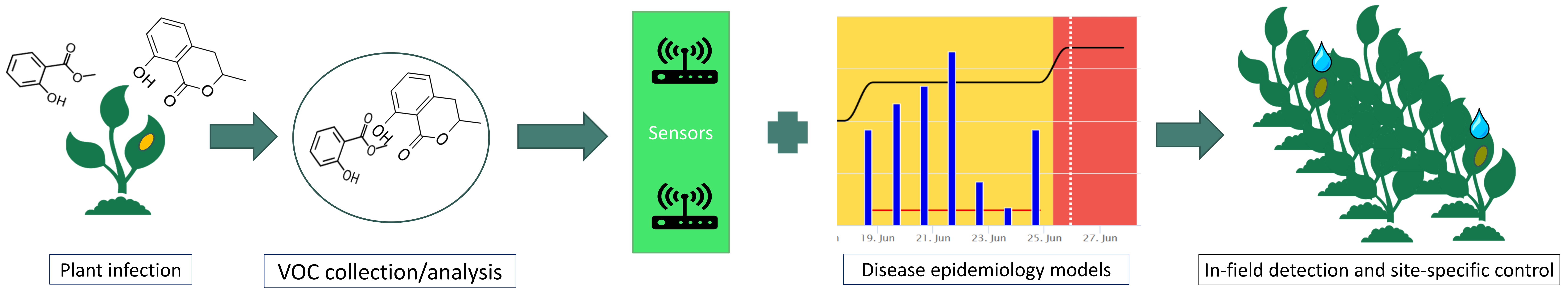
Discussion

Our results showed that specific VOCs emitted from wheat plants can be used to classify plants into healthy and diseased sample groups. Mellein is a compound produced by *P. nodorum* itself and would be a reliable indicator for SNB infection. However, concentration of this compound was very low and it could only be detected when SNB infection was high (25%). At that severity the disease would be visible to the unaided eye in the field anyway and any control intervention might be too late. Interestingly, 4-Methylacetophenone and Z-3-Hexenylacetate were only produced when there was no visible disease present on the plant and α-Cedrene and (E)-b-Ocimene were negatively correlated with SNB infection severity. These compounds could be used in a negative prognosis approach to map the healthy area in a field, where no fungicide application is needed.

Site-specific disease management would greatly benefit from a combination of disease prediction in time and space. Highly sensitive VOC-based sensors that can capture very low concentrations of target volatiles could be used in a time efficient manner, once weather-based epidemiology models have identified the time window of high infection risk. The combination of disease specific sensors and disease epidemiology models would greatly increase the efficiency and accuracy of remote sensors and site-specific disease management (Figure below).

Plant Pest Prevention through technology-guided monitoring and site-specific control (PurPest):

PurPest is a 4-year research and innovation project funded by the EU (2023-2026) to develop, validate and demonstrate a sensor platform to rapidly detect and stop pests and diseases during import and in the field. The sensor concept is based on detection of pest-specific VOCs emitted by host plants invaded by one or several pests. PurPest will determine the VOC signature of plants attacked by *Phytophthora ramorum*, the Fall armyworm (*Spodoptera frugiperda*), the Cotton bollworm (*Helicoverpa armigera*), the Brown marmorated stinkbug (*Halyomorpha halys*) and the Pinewood nematode (*Bursaphelenchus xylophilus*) under different abiotic stress conditions.



Plant infection

VOC collection/analysis

Disease epidemiology models

In-field detection and site-specific control

Reference: Ficke A, Asalf B, Norli HR. 2022. Volatile Organic Compound Profiles From Wheat Diseases Are Pathogen-Specific and Can Be Exploited for Disease Classification. Front Microbiol. 2022 J doi: 10.3389/fmicb.2021.803352..

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