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Commentary: Is *Trichoderma* ear rot on maize really a new dangerous plant disease?

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A Commentary on

Is Trichoderma ear rot on maize really a new dangerous plant disease?

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Introduction

The above opinion paper strongly refers to a publication on pathogenic *Trichoderma* strains causing a novel disease in maize, *Trichoderma* ear rot (Pfordt et al., 2020). As the main findings and conclusions in this study are questioned by the authors, we herewith present a response to their criticisms.

Response to the criticism of weak evidence for *Trichoderma afroharzianum* as an aggressive plant pathogen

The assertion made by Trillas et al. that there is "weak evidence" for the involvement of *Trichoderma afroharzianum* as an aggressive plant pathogen overlooks the substantial experimental data presented in our study. We demonstrated that *T. afroharzianum* can cause significant ear rot in maize, and our findings do not rely on speculative mechanisms of pathogenicity but are grounded in empirical evidence. Our experiments showed clear pathogenic effects under controlled conditions, where *T. afroharzianum* was the sole inoculated agent, leading to pronounced disease symptoms and substantial damage to maize ears. Moreover, the aggressiveness of *T. afroharzianum* was evident in the disease symptoms observed in maize as well as wheat and barley (Pfordt et al., 2023), with significant differences in dry matter loss compared to control groups.

Response to the doubts concerning *Trichoderma afroharzianum* as a primary pathogen

The criticism raised by Trillas et al. suggesting that *Trichoderma afroharzianum* acts only as a secondary invader after Fusarium infection and not as a primary pathogen. We rigorously followed Koch's postulates to confirm *T. afroharzianum* as a primary causal agent of ear rot in maize. The pathogen was isolated from infected maize ears in the field, cultured, and successfully re-inoculated on healthy maize plants, leading to the same disease symptoms.

Unlike findings reported in other studies where *Trichoderma* was identified as a symptomless secondary fungus following *Fusarium* infections, our results revealed that *T. afroharzianum* induced distinct, strong symptoms caused by its own infection. Unlike opportunistic species that require external damage or co-infection to establish themselves, *T. afroharzianum* was able to infect maize ears directly, as observed in our silk channel inoculation experiments. This finding is crucial, as it differentiates *T. afroharzianum* from other species of *Trichoderma* traditionally considered as opportunistic or secondary pathogens (Pfordt et al., 2024a).

Furthermore, the high disease severity and dry matter loss observed in our experiments, compared to the controls, strongly indicate that *T. afroharzianum* has aggressive pathogenic characteristics (Pfordt et al., 2024b). Not least, we refer to recent reports from Northern Italy on *Trichoderma* ear rot detected in commercial maize fields (Sanna et al., 2022).

Response to the criticism regarding environmental and agronomic conditions

Trillas et al. raised concerns about the lack of detailed environmental and agronomic conditions described in our study, particularly regarding temperature, humidity, and pest management practices. While our publication did provide relevant information on the warm and dry summer conditions under which *Trichoderma afroharzianum* ear rot was observed in maize fields, it is important to emphasize that the focus of our study was on establishing the pathogenicity of *T. afroharzianum* rather than conducting an epidemiological analysis of environmental factors. These conditions were sufficient to establish the context of the outbreak and demonstrate the potential of *T. afroharzianum* as a primary pathogen.

In addition, our greenhouse experiments, which followed standard protocols, were designed to minimize the impact of external environmental variables, allowing us to focus on the pathogenic potential of the fungus. While the field conditions may vary and contribute to disease severity, the controlled conditions in the greenhouse (23°C) ensured that the results were attributable solely to the pathogen and not confounding environmental factors. The following publications further support the findings of *T. afroharzianum's* pathogenic potential under controlled environmental conditions:

Pfordt et al., 2023: Pathogenicity of *Trichoderma afroharzianum* in cereal crops.

Pfordt et al., 2024a: Phylogenetic analysis of pathogenic and non-pathogenic *Trichoderma* isolates.

Pfordt et al., 2024b: Impact of *Trichoderma afroharzianum* infection on fresh matter content and grain quality in maize.

Response to concerns regarding the use of high conidia concentrations

Trillas et al. have criticized our study for employing a spore concentration of 10^6 conidia/mL of *Trichoderma afroharzianum*, suggesting that such high concentrations are unlikely to occur in natural environments and lack justification in the literature. However, it is crucial to highlight that the use of a concentration of 10^6 conidia/mL is standard practice not only in our research but also in pathogenicity assays for other pathogens, such as *Fusarium* and *Aspergillus*, where similar inoculation strategies are employed (Reid et al., 1995; Reid and Hamilton, 1996)

This concentration allows for a robust evaluation of the pathogen's virulence under controlled conditions and in field studies. Furthermore, Trichoderma-based biocontrol products are also commonly applied at similar concentrations to plants in commercial settings (Lombardi et al., 2020; MycoSolutions AG, 2022; Du et al., 2024).

Response to concerns regarding the specification of maize hybrids

Trillas et al. have raised the concern that we did not specify the maize hybrids used in our greenhouse experiments, referring only to "maize seeds of two varieties." While we acknowledge the importance of transparency in methodology, it is important to clarify that our primary objective was to assess the pathogenicity of *Trichoderma afroharzianum* isolates rather than to compare the susceptibility of different maize hybrids.

In our greenhouse experiments, we utilized the maize hybrids LiKeit (DSV) and Mallory (LimaGrain). These hybrids were selected for their widespread use in commercial maize production, ensuring that our findings would have practical relevance across commonly cultivated varieties.

The use of two maize varieties was intended to provide a broader basis for our pathogenicity assays. Both hybrids were selected based on their common use in agricultural practice, ensuring that our findings could be applicable to a broader range of maize cultivars used in practical production.

Discussion

Altogether, the case of Trichoderma ear rot demonstrates the ever-known potential of novel pathogens to evolve as a result of adaptive evolution. Modern crop production systems with the continuous cultivation of a limited number of crop species pose a constant challenge to microbial populations in the agroecosystem. Adaptive evolution has been experienced in the past by adaptation to new hosts of formerly unimportant pathogens or pests, which suddenly gain significance in threatening particular crops. All these phenomena indicate that pathogen and pest evolution continue and we can never relax with the situation as given at a present point of time. The crop protection scientific community should be alert and open-minded towards such developments in order to keep pace with upcoming novel challenges in crop health. Trichoderma cob rot is a striking novel example of such natural evolutionary processes and thus a very interesting case to study, in order to better understand the fundamental factors and principles underlying the evolution of pathogenicity and parasitism on plants. Moreover, it raises our awareness of risks associated with the use of living microbes in crop protection.

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AP: Writing – original draft, Writing – review & editing. AT: Writing – review & editing.

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