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In vivo experiments on the flow of mycotoxins in plants

Maria Cavallero[1], Enrico Rolli[1], Francesca Degola[1], Chiara Dall'Asta[2]

[1] Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Via Università 12, 43121, Parma, Italy
[2] Department of Food and Drug, University of Parma, Parco Area delle Scienze 27/A, 43124, Parma, Italy

INTRODUCTION & AIM

Mycotoxins are secondary metabolites produced by some fungal species.
There exist over 300 varieties of these toxins (Juraschek, et al., 2021), predominantly concentrated in agricultural regions, as the primary hosts for these fungi are specific crops such as cereals, grapevines, coffee, and fruits. It should be noted that these toxins are pervasive in all products derived from these cultivated sources.
Climate change is progressively extending the geographical regions affected by the proliferation of certain pathogenic species towards higher latitudes, elevating the risk of contamination in areas that were previously unaffected by this issue.
Renowned for their adverse impact on both human and animal health, these compounds have garnered extensive attention in terms of biosynthesis, detection, health risks, and mechanisms of action. Despite rigorous study, the fate of mycotoxins, once liberated into plant tissues following fungal infection, remains incompletely understood.
Nevertheless, the plant employs a defense mechanism, consisting of a non-specific enzyme pool to modifying these xenobiotics, preventing them from interfering with its primary metabolism.
This process takes place in three stages (Righetti, et al., 2019):
- phase I: structural modification
- phase II: conjugation, incorporating processes such as glycosylation or hydroxylation
- phase III: storage, in which the transformed 'masked mycotoxin' is placed inside the vacuole or cell wall.

This process brings about a transformation in the chemical characteristics of the initial toxin. While it might result in a modification of toxicity, it may also induce alterations in polarity and water solubility.
Three mycotoxins, Zearalenone (ZEN), T-2, and Aflatoxin B1 (AFB1), differing in chemical characteristics, were selected for each of the experiments.
It is precisely due to this last change that our focus centers on investigating the uptake and translocation of these toxins within plants.
As already described, it is an issue that is mainly related to food safety, and for this reason we have chosen *Zea mays* L. as a model system.
Two types of laboratory experiments were set up: i) a 'split root' experiment in which the toxins were added individually to the medium in contact with the half root apparatus; ii) a dipping experiment in which the leaf, once scarified, was dipped in a toxin containing solution.

METHOD

The toxins chosen is Aflatoxin B1 (AFB1), it was prepared by dissolving 100µg compound in 50ml of Murashige and Skoog liquid medium with an addition of 6µl dimethylsulphoxide (DMSO).
For both experiments, the surface of the maize seeds was sterilised with ethanol and sodium hypochlorite and placed on ¼ MS agarized medium contained in glass jars.


Figure 1

When the plant was developed enough to be handled (7-10 days), it was treated for the split root experiment (Figure 1).
The root system was divided into two portions, and each portion was placed in a glass test tube filled with a 1/2 concentration of Murashige and Skoog culture medium.
After about 2 weeks, both portions grew to the same mass the medium was replaced: the portion to which toxin was added was called 'Donor (D)' and the portion to which new medium (1/2 MS) was added was called 'Receiver (R)'.
Samples of the two media (D and R) were taken at time 0, 7 and 14 days, and on day 14 the plant portions were also sampled: the leaves and the 'D', 'R' root portions.

For the second set of experiment (Figure 2) the plants were sown as described above and left to grow in pots, waited until the third leaf was well developed. A portion of approximately 1.5 cm in the central part of the third leaf was chosen and wetted with distilled water to simplify scarification with 600 grit sandpaper (Campbell et al., 1999), after which the leaf was placed in a Petri dish, previously modified (Figure 3), inside which the toxin solution was placed, taking care that the leaf was well immersed.


Figure 2

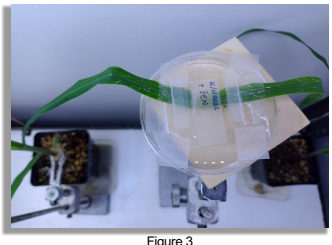
Figure 3

The average sampling was carried out at times 0, 7 and 14 days. At the end of the experiment (day 14), plant material (treated leaf, distal leaf, roots) was sampled for analysis using IMS-qTOF.

RESULTS & DISCUSSION

✓ Through the "split-root" experiment, our goal was to illustrate the plant's ability to transport the toxin internally. Analysis reveals (Figure 4 A) that the "D" segment efficiently absorbed the toxin (AFB1) from the medium within a span of 14 days. Moreover, in line with this observation (Figure 4 B), a corresponding peak signifies the presence of the AFB1 toxin in the aerial part of the plant (leaves). These findings echo earlier *in vitro* studies on maize plants, reinforcing the evidence of metabolites derived from the mycotoxin AFB1.

The robustness and innovation of this experiment come to fruition through the scrutiny of the radical "R" segment. Figure 4 C, clearly illustrates the presence of unmodified AFB1 in the Recipient portion, even though it was not in direct contact with the toxin-containing solution.

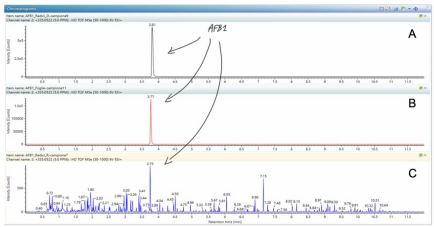

Figure 4

This experimental evidence conclusively establishes the capability of the AFB1 toxin to move within the corn plant.
In summary, it can be inferred that unaltered AFB1 exhibits a degree of mobility within the corn plant.

However, to ascertain the specific mechanisms involved, further analyses are imperative. These investigations should aim to elucidate whether the mobility occurs through vascular transport, utilizing xylem for upward movement and phloem for downward transport, or through processes such as apoplastic or symplastic diffusion. Clarifying these details will provide a comprehensive understanding of the AFB1's internal movement within the plant.

✓ The objective of the second experimental set was to ascertain the potential internal mobility of the toxin upon inoculation into a leaf. The analyses distinctly indicate the presence of the AFB1 toxin in its unmodified form at the site of administration (Figure 5 A).

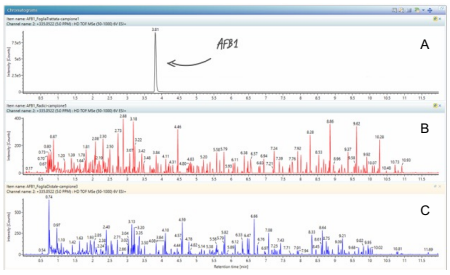

Figure 5

This observation implies that when administered at the foliar level, the toxin fails to exhibit mobility within the plant. In other words, the toxin does not distribute itself effectively beyond the point of application on the foliage.
In this instance, in addition to the treated leaf, the roots and the distal leaf were also analysed. The findings, depicted in figure 5 B and C, reveal the absence of the AFB1 toxin in these portions.

CONCLUSION

In conclusion, the split-root experiment stands as a robust and effective method for investigating the mobility of toxins within plants. This experimental approach provides valuable insights into the internal moviment of substances, offering a contribution to our understanding of plant physiology and toxin movement.
On the contrary, the experiment involving toxin administration through the leaves requires refinement to delve more deeply into the underlying factors contributing to the observed lack of toxin mobility.

FUTURE WORK / REFERENCES

A key direction for future research is to use other mycotoxins with different chemical characteristics, and also to explore the localization of toxins through microscopy and imaging techniques.

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