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Effects of Seed Treatment with Systemic Fungicides on Fungal Endophytes

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Seed treatment with systemic fungicides is a firmly fixed practice for most of the agricultural crops. The seed treatment is intended to protect the crop against seed as well as soil-borne diseases. Endophytes are ubiquitously present in plants and contributing to plant growth and development besides offering resistance to biotic and abiotic stresses. In seeds, endophytes may play a role in seed development, germination, seedling establishment and overall crop performance, which is transmitted vertically from seed to seed as in many grasses and/or acquired horizontally from the soil and the environment. At present, there is increasing evidence that fungicidal applications to manage diseases might inadvertently also affect endophytes. Use of systemic fungicides as seed treatment may not only affect the seed endophytes but also their attendant benefits to seedling growth and establishment. While, foliar applications also affect foliar endophytes. Reinforce the importance of seed endophytes to seedling growth and establishment and draw attention on how to trade the balance between the benefits of seed treatments and the direct and indirect costs incurred due to loss of endophytes. Reduced-risk fungicides and identifying fungicide-resistant endophytes are suggested to sustain the endophyte contribution to crop performance.

Definition of 'endophyte'

Endophytes definition proposed by (Hallmann *et al*. 1997), as 'those microorganisms that can be isolated from surface-disinfected plant tissue or extracted from within the plant, and that do not visibly harm the plant'. However, this definition is not perfect as, first, it does not account for endophytes that are unculturable and, second, it is not always easy to assess phytopathogenicity and distinguish latent pathogens from endophytes, particularly for non-culturable fungi that are part of the microbiome community. Fungal endophytes exhibit a range of symbiotic relationships with their hosts as well as various lifestyles; for example, some of these interactions can be mutualistic in which the long-term relationship is beneficial to both partners (Card *et al*. 2016). In addition, some endophytes may only exhibit a mutualistic interaction for one plant species, but not for another (Hardoim *et al*. 2015). However, the endophytes are microorganisms that can be detected inside healthy plant tissues and are asymptomatic. A microorganism has an endophytic lifestyle, it must be demonstrated that it can be successfully reintroduced into disinfected seedlings as judged by microscopy, thereby fulfilling Koch's postulates (Hyde and Soytong 2008).

Role of endophytes in ecosystem

Fungal endophytes play crucial roles in ecosystems by protecting plants against many biotic and abiotic stresses, increasing their resilience, and helping plants to adapt to new habitats. Biotic stresses from which endophytes can provide protection include plant pathogens, insects and nematodes. Abiotic stresses include nutrient limitation, drought, salination and extreme pH values and temperatures. In return, plants provide spatial structure, protection from desiccation, nutrients and, in the case of vertical transmission, dissemination to the next generation of hosts. Endophytes may also play a role in the ecosystem by affecting plant growth through antagonistic fungal-fungal interactions. An example is the interaction between the pathogen *Ustilago maydis* and the endophyte *Fusarium verticillioides* within their shared plant host (maize, *Zea mays*), whereby the endophyte is capable of reducing the rate of pathogen growth, possibly by secreting metabolites that break down plant compounds that limit *U. maydis* growt. Some fungal endophytes in ecosystems may be to initiate the biological degradation of a dead or dying host plant which starts the process of nutrient recycling. Dominate endophyte taxa of grasses are also found as common airborne fungi. They speculate that the transition from an endophyte to a saprobe requires sporulation after host senescence for some of these species to complete their life cycle and being established in tissues, endophytes have immediate access to plant nutrients available during plant senescence.

In light of the increasing evidence of the role of endophytes on plant growth and stress tolerance, their use in agriculture could be constrained by the practice of seed treatment with fungicide. Although seed treatment includes application of fungicides, insecticides or rodenticides, the majority of seed treatment is with fungicides. Seed treatments are essential condition for managing diseases to increase stand establishment, seed yield and quality. While the major aim of seed treatments with fungicides is to bring down the pathogen load on the seed surface or inside without affecting seed viability and seedling fitness. Since environmental filtering and maternal factors determine the constitution of the fungal microbiome in seed, the effects of seed treatment with systemic fungicides on the seed endobiome and their consequences on seed and seedling performance need to be addressed.

Case studies

Since endophytes are inextricably embedded in the plant tissue, unravelling their role in seedling growth is problematic. Nevertheless, several studies have attempted to cleanse the seed using systemic fungicides to examine the effects thereof on seedling growth attributes. In recent years, there is mounting evidence to suggest that foliar application of fungicides significantly affects non-target organisms, such as the endophytic fungi. Fungicide application on wheat plants leads to significant differences in relative abundance and diversity of non-target fungi (Karlsson et al., 2014) and also inhibited the growth of endophytic yeast and filamentous fungi (Wachowska et al., 2013). Batzer and Mueller (2020) reported differential effects of fluxapyroxad and pyraclostrobin sprays on *Diaporthe* and *Alternaria* endophytes; the fungicides significantly increased the proportion of endophyte species belonging to *Diaporthe* but decreased those of *Alternaria*. Besides affecting endophytic fungi, application of foliar fungicides and other plant protectants also reduce the endophytic proteobacteria (Chen et al., 2020).

Though less documented, seed treatment with fungicides could lead to similar loss or disruption of the seed microbiome including the endophytes compromising seed germination and early seedling development (Lugtenberg et al., 2016). For example, in rye grass and tall fescue, seed treatment with fungicides reduced endophyte loads by over 60 per cent (Leyronas et al., 2006). Seedling endophyte abundance in rye grass was always higher when no fungicides were applied (Hill and Brown, 2000). Fungicide application reduced the vertical transmission of *Neotyphodium* endophyte AR37 into germinated seedlings of rye grass (Chynoweth et al., 2012), although Cruz et al. (2018) reported no detrimental effect of fungicide application on AR37 endophyte content in rye grass seeds. Thus, whether through foliar application, as most studies have demonstrated, or through seed treatments, there is evidence to suggest that fungicides adversely affect the endophyte load and composition of plants and seeds, possibly impairing the ecological fitness of plants (Nettles et al., 2016).

Vasanthakumari et al. (2019) examined the effect of pre-sowing fungicidal treatment on seedling growth of rice, green gram, soybean and cowpea. In all these crops, treatment with 0.2% bavistin eliminated the endophytes and reduced seedling growth and vigour compared to untreated seed in the absence of disease. The reduced seedling growth in rice was partially restored upon enrichment of the seedlings by a consortium of endophytes obtained from untreated seeds. These results strongly suggest that the decrease in seedling growth upon fungicide treatment is due to the loss-of-function associated with the endophytes, rather than to the phytotoxicity of the fungicide. In another study, Puente et al. (2009) found that seedling establishment was impaired in cactus seeds disinfected with antibiotics. Inoculation of antibiotic-treated cactus seedlings with bacteria isolated from control seeds restored seedling vigour. The results of a few studies allow us to speculate on the negative effects of treating seeds with systemic fungicides. The significant reduction of germination in bavistin- and thiram-treated wheat seeds was due of their inability to mobilize stored starch in the absence of endophytes (Gogna et al., 2015). The increased propensity of citrus, banana and leather leaf fern for infection by virulent pathogenic strains after application of benolate could be because of the lowered defences in the absence of endophytes (Kloepper et al., 2013). Though direct evidence is lacking, the results obtained for leaf tissues in this regard bolster such a hypothesis. Mango leaves treated with hexaconazole, a broad spectrum triazole systemic fungicide, became infected by FE species that could not infect untreated leaves (Mohandoss and Suryanarayanan, 2009).

Since fungicides have direct effects on plant metabolism, all fungicide-induced effects cannot be attributed to the elimination of endophytes by the chemicals. It is known that high concentrations of fungicides can disrupt plant metabolism. Storing seeds after treatment with fungicide for long periods can result in phytotoxicity (Lamichhane et al., 2020). Similarly, at higher concentrations, benomyl inhibits root mitotic activity (Dane and Dalgic, 2005). Many fungicides reduce root nodule development (Martensson, 1992) and reduce the development of mycorrhizal fungi (Menge, 1982).

Conclusion

Management of seed- and soil-borne pathogenic fungi using fungicides is important for ensuring food security. It is only recently that the crops have been recognized as a *holobiome* consisting of the plant and all its associated microbes. This has led to the suggestion of conserving seeds along with their associated microbes, such that these microbes are not lost forever due to the global practice of seed treatment. We reviewed the trade-off of pre-sowing seed treatment in defending seeds against seed- and soil-borne pathogens on the one hand and the possibility of losing seed benefiting endophytes on the other. Considering the potentially important role of seed-borne endophytes in seed germination and seedling growth, and being a source of endophyte inoculum for the different tissues of the developing plant, the century-old practice of routine seed treatment should be revisited. The gain accrued by seed treatment in disease management versus the potential loss in crop performance due to disturbance of seed endobiome by seed treatment should be studied for more crops using fungicides exhibiting different modes of action.

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