

Arbuscular mycorrhizal fungi coupled with *Epichloë* endophytes reshaped the rhizosphere microbiome structure and induced synergistic resistance to leaf spot disease in perennial ryegrass

Youlei SHEN^{1,2} and Tingyu DUAN^{1,2*}

¹State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, Lanzhou University, Lanzhou 730020, China

²College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, 730020, China

*Corresponding author: duanty@lzu.edu.cn

Perennial ryegrass (*Lolium perenne*) frequently forms simultaneous associations with arbuscular mycorrhizal (AM) fungi and *Epichloë* endophytes, residing belowground and aboveground of host plants, respectively. These beneficial symbionts enhance plant stress resistance by regulating nutrient absorption, increasing the activity of defensive enzymes and hormone levels, and upregulating stress-related gene expression. The rhizosphere microbiome, often referred to as the plant's 'second genome', plays a crucial role in influencing productivity, and resilience to stress. While AM fungi and *Epichloë* endophytes are known to independently alter rhizosphere microbial communities, their interactive effects on rhizosphere microbiome, particularly under pathogen stress, remain poorly understood and underexplored.

A greenhouse experiment was conducted to investigate the individual and interactive influences of the AM fungus (*Acaulospora delicata*) and *Epichloë* sp. LpTG-3 strain AR37 on defence physiology (enzymes and hormones), and rhizosphere microbiome-metabolite profiles related to resistance against *Bipolaris sorokiniana*, responsible for leaf spot disease.

When applied alone, *A. delicata* reduced disease incidence by 38% and disease index by 57%, while *Epichloë* strain AR37 alone decreased disease incidence by 26% and disease index by 44%. Co-colonisation of *A. delicata* plus AR37 synergistically amplified these effects, achieving a 54% reduction in disease incidence and a 76% reduction in disease index, with *A. delicata*

playing a predominant role in pathogen suppression. The tripartite interaction significantly enhanced defensive enzyme activities (superoxide dismutase, peroxidase, catalase), which scavenge pathogen-induced ROS, while reducing malondialdehyde (MDA) content by 29%, indicating mitigated lipid peroxidation. Notably, the *A. delicata*-*Epichloë* AR37 interaction increased ethylene levels by 11%, a critical shift linked to defence priming. *A. delicata* also increased the alpha diversity of both the rhizosphere bacterial and fungal communities, in contrast to the minimal microbiome alterations observed from AR37 or pathogen alone. In the presence of the phytopathogen *B. sorokiniana*, the interaction between *A. delicata* and AR37 enriched fungal taxa within the division Basidiomycota and the bacterial phyla within the Acidobacteria, Actinobacteria, and Candidatus Sumerlaeota, while simultaneously suppressing the dominance of the fungi within the division Ascomycota. Further analysis revealed that the relative abundance of Basidiomycota, Acidobacteria, Actinobacteria, and Candidatus Sumerlaeota was negatively correlated with the disease index and positively correlated with MDA and ethylene levels in ryegrass. Additionally, rhizosphere metabolomics identified four pathogen-inhibitory compounds that significantly accumulated in the multisymbiont systems: (benzo(b)thiophene-6-yl) acetic acid, erucic acid, acetamide, and dodecanoic acid. The concentrations of these metabolites were negatively correlated with the disease index and positively correlated with the relative

abundance of some taxa. *In vitro* experiments indicated that acetamide exhibited a pathogen inhibition rate of 52%.

Our findings demonstrate that the synergistic effects of *A. delicata* and *Epichloë* sp. strain AR37 stimulated

the production of rhizosphere metabolites, such as (benzo(b)thiophene-6-yl) acetic acid, acetamide, which may have recruited a beneficial microbiome to the host rhizosphere to strengthen anti-disease defences.