Friedrich Schiller University, Institute of Microbiology - Microbial Communication, Jena, Germany

The parasitic-neutral-mutual continuum of plant-fungal interactions

Oluwatosin Abdulsalam, Erika Kothe, Katrin Krause

Summary

Interactions of land plants with fungi are of imminent importance to crop production and thus for human nutrition. However, interactions range from pathogenic fungi, e.g. cereal rusts, to beneficial inter-actions with plant growth promotion through soil, endophytic or mycorrhizal fungi. Thus, mutually beneficial, neutral or parasitic/pathogenic interactions can be distinguished. In order to identify more general mechanisms on the fungal side coping with environ-mental and plant response associated stress, modern technologies are available including the -omics technologies. In addition to that, dif-ferent interactions may be compared, both on a more general level, but also at very small scale to identify the different parameters guiding interchange of nutrients and signals. This will allow for a holistic view on plant health necessary to establish eco-friendly tech-nologies also in crop protection and plant nutrition.

Introduction

Plants have been in association with fungi in their terrestrial envi-ronment millions of years and mutualistic, parasitic or neutral inter-actions have evolved (MAYER, 1989). Neutralism with respect to plant-fungus interactions might be encountered with some endo-phytes. Endophytic merely describes the life style where a fungus is living within a plant host without causing symptoms (for a recent review: KOTHE and TURNAU, 2018). It seems necessary, however, to note that an infection and showing growth *in planta* are essential to assign the fungus an endophytic life-style – mere isolation from plant tissue or DNA-dependent identification from a seemingly healthy, surface-sterilized plant part does not qualify for the verification of an endophytic nature. This latter part, regrettably, is often disregarded in literature.

The outcome such an association, however, is influenced by the environment, resulting in a dynamic range of interaction types. Fungi are known to play major roles in natural ecosystems and in modern agriculture based on their nutritional versatility and various inter-actions with plants (ZEILINGER et al., 2016). They are efficient de-composers, and this ability gives them the capacity to make complex nutrients available to interacting partners, e.g. by mobilization of soil phosphates (GAIND, 2016; ZHANG et al., 2018; ZHANG et al., 2014). The interactions of such plant growth promotion soil fungi with plants is hampered by the plant defense reactions that have evolved against pathogens (STASKAWICZ, 2001). Thus, recognition of benefi-cial partners is essential. The recognition and differentiation of ef-fector or 'epitope-like' cues to differentiate between friends and foes is therefore imperative for the survival of the plant. We therefore aim to discuss the different types of plant-fungal interactions to elucidate underlying mechanisms that govern such interactions and to describe the continuum of parasitic-neutral-mutual interactions.

Plant-fungus interaction types

Different plant parts can form niches for fungi (Fig. 1). The fungi can form mutualistic, neutral or pathogenic interactions with their host
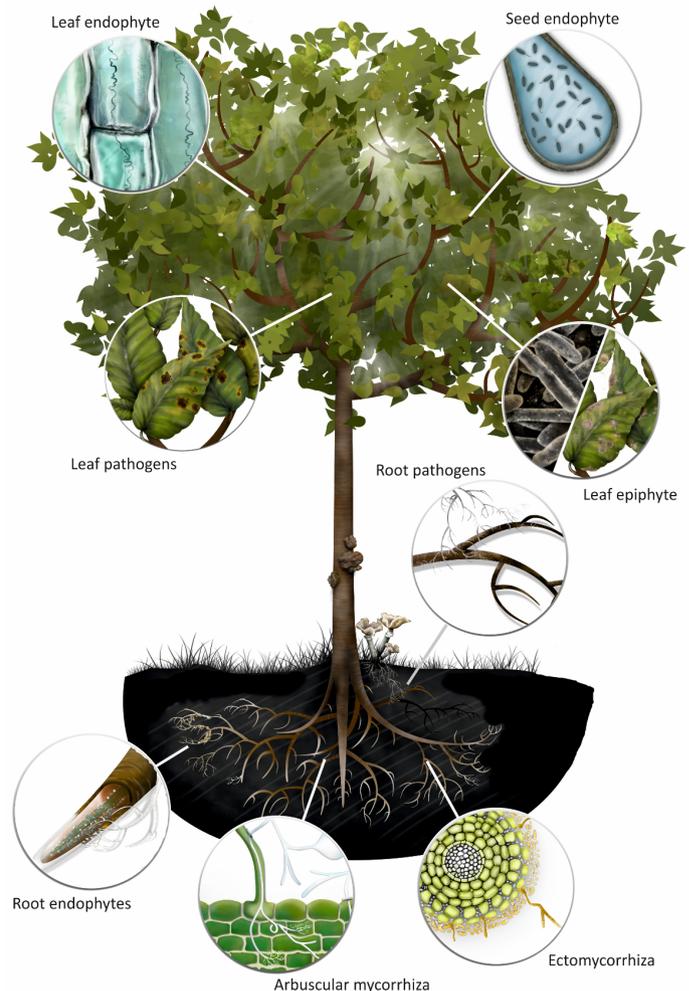
Fig. 1: Plant interactions with mutualistic, pathogenic and neutral fungi in different tissues.

plant. Mutualism as the outcome of plant-fungal interaction refers to an interaction where both partners have a positive net pay-off as a result of their interaction. A classic example of plant-fungal mu-tualistic interactions is the mycorrhiza, where fungi supply water and minerals while the plant provides the sugar for fungal growth (e.g., HENKE et al., 2015; PARNISKE, 2008; WAGNER, et al., 2015). This well-studied interaction (MARTIN et al., 2017) can be clearly separated from plant endophytes, where mutualism is not really ob-vious (BAMISILE et al., 2018; CLAY and HOLAH, 1999; DENG and CAO, 2017).

Unlike mycorrhizal roots, endophytic fungi show less frequently morphological changes in association with their host, and they are not necessarily restricted to the plant root. The neutralism observed with some endophytes merely describes a life style, where a fungus is living within a plant host without causing symptoms (for a recent

review: KOTHE and TURNAU, 2018). It seems necessary, however, to note that an infection and showing growth *in planta* are essential to assign the fungus an endophytic life-style – mere isolation from plant tissue or DNA-dependent identification from a seemingly healthy, surface-sterilized plant part does not qualify for the verification of an endophytic nature. This latter part, regrettably, is often disregarded in literature.

Parasitic interactions, where one partner benefits at the detriment to the other, are the most prominent type of fungus-plant interaction. Many pathogens are known to exploit plants in the attempt to gain access to nutrition, and often they are tissue specific, like *Phytophthora palmivora* infecting buds and seeds (CARELLA et al., 2018) or *Heterobasidion annosum* leading to root rot (LUNDÉN et al., 2015). Other fungal infections (for example, the smut fungus *Urocystis trientalis*) are known to be systemic (PIQUERAS, 2001). Some parasitic fungi have been shown to alter specific metabolic pathways in the plant host, leading to repression of pivotal compounds necessary for host physiology. A good example is the biotrophic pathogen *Gymnosporangium asiaticum* that represses amino acid/nucleotide metabolism in Rosaceae due to a deficiency in some precursor sugars. This repression, in turn, results in the systemic symptoms like cell wall synthesis and lesion repair (LEE et al., 2016). Mechanistic studies like this explain the systemic effects of infections, even though the site of infection was tissue specific.

Molecular basis for different types of interactions

For a successful interaction, regardless of the outcome, there is a need for communication and/or signaling between the interacting pair (MBENGUE et al., 2016). Apart from chemical cues and small peptide signaling compounds, a small class of proteins called effectors become increasingly addressed. They are encoded in genomic regions that are rarely expressed when the organism is grown axenically, but show high induction in interactions and show efficient delivery into the plant cells (SELIN et al., 2016). They have been shown from pathogenic, endophytic and mycorrhizal interactions (KEMEN et al., 2015). A good example for the latter are arbuscular mycorrhizal fungi, which respond to plant derived strigolactones with effector/elicitor production to suppress the plant defense (VOSS et al., 2018). In ectomycorrhizal fungi, effector proteins (called mycorrhiza-inducing small secreted proteins, MiSSPs) were characterized as well (PLETT et al., 2014). This group of proteins thus shows important functions and potential convergent evolution in different plant-fungus interactions.

Epigenetic switches/abiotic factors causing dynamic interactions

Plant phenotypic adjustment to the external environment is a big factor in the outcome of interaction with its microbiota. The differing response to these stimuli gave rise to dynamics in the interaction. Epigenetic mechanisms involve DNA methylation, histone modifications and histone variants, and small RNAs leading to changes in gene expression (VANNIER et al., 2015). Epigenetics and the plant microbiota play a very important role in the adjustments of the plant to its external environment, they therefore need to be considered in the evolution of interaction and development of the plant as a host (VANNIER et al., 2015). Post-transcriptional and post-translational modifications in addition allow for a quick response to environmental stress (SHAW and ETTERSON, 2012). Epigenetic mechanisms have also been shown in fungus-plant interactions. SOYER and co-workers (2014) showed, how a pathogenic fungus uses histone modification as a switch to induce specific gene expression by lifting chromatin-mediated repression while infecting plant, therefore switching the fungal lifestyle towards pathogenesis (SOYER et al., 2014).

Evolution of plant-fungal interactions

It is believed that arbuscular fungus-plant interactions have evolved over 480 million years since plants occurred on the land surface. This is even more convincing as reports have been showing examples of mutualistic interaction of this class of fungi with plants from very early lineages like liverworts (HUMPHREYS et al., 2010). The similarity in the strategies and structures of fungal pathogens and symbionts like arbuscules and haustoria are seen with both, developing into a structure with host cell invagination enabling efficient exchange of nutrients as well as regulatory molecules. Many arbuscular mycorrhizal fungal genes, however, are not only shared with pathogens, but with ectomycorrhizal fungi as well. Mycorrhiza-specific genes, and especially those encoding effectors, have been shown to evolve rapidly (CORRADI and BONFANTE, 2012), and the diversity of the effectors contributes to host range and parasite speciation (DODDS, 2010). Evolution of host specificity has been well studied in fungal endophytes. It is believed to originate from the close adaptation between the host plant and its fungal partner, suggesting a mutual influence of co-habitation and co-evolution (RAI and AGARKAR, 2016). During long-term association, this partnership becomes stronger and permanently imprinted in the genetic constitution of both partners, which finally develops into complementary genetic system (MORICCA and RAGAZZI, 2008). The co-evolution phenomenon tentatively explains the lack of plant defense reactions against the presence of microbial endophytes (CHRISTENSEN et al., 2002) and the ability of endophytes to produce bioactive metabolites usually known from host plants (ZHAO et al., 2011).

Impact of plant-fungal interactions in the ecosystem

Fungi are very successful inhabitants of the soil environment. Spores can survive extremely harsh conditions and germinate into actively growing mycelia when the conditions become favorable. The batteries of lytic enzymes and mechanical intrusive growth allow them to access and utilize rather complex organic nutrients. This feature helps in the breakdown of complex nutrient sources that other community members may only then utilize. Therefore, proliferation of fungal community members results in a burst of microbial community diversity making fungi keystone species in ecological communities. One specific example is a fungal species reported to increase fecundity and biomass of North American cheat grass, leading to higher probability of fire and therefore a frequent change in the ecosystem structure and function (BAYNES et al., 2012). The fungus most importantly increases the probability of the survival of cheat grass seed, ensuring a good invasion strategy of cheat grass in North America. A fungal keystone species is more evident in pathogenic fungus, as obvious drastic effect is observed with their presence and therefore leads lead to change in the macro- and micro-environment, having significant impact on the ecosystem processes. Another, pathogen example is *Ophiostoma ulmi* that, as a single fungal species, changed ecosystem properties of forests in Europe and Northern America (for review: TAERUM, 2018).

Approaches in plant-fungal research

Advancement in genetics, proteomics and analytical chemistry has gone a long way to help unravel many of the molecular and chemical basis of interaction between plants and fungi. For the fundamental classification of interactions, it has been difficult to clearly verify the type of interaction in an interacting pair. This result of dynamics in biological interactions show the importance of a holistic view on the allocation of resources and cost accrued with the interaction. Approaches used to understanding the basis of biological interactions include proteomics and/or protein assays involved in, e.g., sugar transport with the SWEET family (CHEN et al., 2010; ECKARDT,

2011; Ge et al., 2008; Harrison, 1996), ATPase activity to characterize interaction sites (Lei and Dexheimer, 1988), or genetic analyses with techniques like RT-qPCR and RNAseq to identify a wider range of inducible genes. Biological interactions limited to specific tissues may be studied with the advancement in sectioning, e.g. cryosectioning of root tissues used in mycorrhizal studies. This now needs to be combined with other techniques like *in situ* probing for a detailed analysis of interaction structures to assess signals and proteins at the site of interaction. Spectrometric and spectroscopic methods have allowed the detection and identification of small molecules and enzyme activities that play key roles in biological interactions (Christensen and Kolomiets, 2011). Developments in laser microdissection have gone steps further to afford researchers possibilities in studying tissue samples (Day et al., 2005), especially for gene and protein expression studies. However, they may also be used for chemical or structural analyses. Since tissue samples still contain living cells, chemical labeling may be used within a tissue sample to the understand biosynthesis and exchange of molecules in the interaction with radioactive label or stable isotopes. It seems mandatory now to tackle the utilization, metabolism or transfer of signaling molecules to characterize the function of these molecules in regulating the interaction. Only such a broader understanding will yield targets that allow for the development of more specific plant protection compounds on the one hand, while on the other hand signals providing better plant growth may be used to overcome the current strategy of over-fertilization. In terms of a more healthy environment, these two measures are affording a better human health while providing food for an increasing population.

Acknowledgements

References

Bamisile, B.S., Dash, C.K., Akutse, K.S., Keppanan, R., Wang, L., 2018: Fungal endophytes: Beyond herbivore management. Frontiers Microbiol. 9, 544. DOI: 10.3389/fmicb.2018.00544

Baynes, M., Newcombe, G., Dixon, L., Castlebury, L., O'Donnell, K., 2012: A novel plant-fungal mutualism associated with fire. Fungal Biol. 116, 133-144. DOI: 10.1016/j.funbio.2011.10.008

Carella, P., Gogleva, A., Tomaselli, M., Alfs, C., Schornack, S., 2018: *Phytophthora palmivora* establishes tissue-specific intracellular infection structures in the earliest divergent land plant lineage. Proc. Natl. Acad. Sci. USA 115, E3846-E3855. DOI: 10.1073/pnas.1717900115

Chen, L.-Q., Hou, B.-H., Lalonde, S., Takanaga, H., Hartung, M.L., Qu, X.-Q., Guo, W.-J., Kim, J.-G., Underwood, W., Chaudhuri, B., Chermak, D., Antony, G., White, F.F., Somerville, S.C., Mudgett, M.B., Frommer, W.B., 2010: Sugar transporters for intercellular exchange and nutrition of pathogens. Nature 468, 527-532. DOI: 10.1038/nature09606

Christensen, M.J., Bennett, R.J., Schmid, J., 2002: Growth of *Epichloë/Neotyphodium* and p-endophytes in leaves of *Lolium* and *Festuca* grasses. Mycol. Res. 106, 93-106. DOI: 10.1017/S095375620100510X

Christensen, S.A., Kolomiets, M.V., 2011: The lipid language of plant-fungal interactions. Fungal Genet. Biol. 48, 4-14. DOI: 10.1016/j.fgb.2010.05.005

Clay, K., Holah, J., 1999: Fungal endophyte symbiosis and plant diversity in successional fields. Science 285, 1742-1745. DOI: 10.1126/science.285.5434.1742

Corradi, N., Bonfante, P., 2012: The arbuscular mycorrhizal symbiosis: Origin and evolution of a beneficial plant infection. PLOS Path. 8, e1002600. DOI: 10.1371/journal.ppat.1002600

Day, R.C., Grossniklaus, U., Macknight, R.C., 2005: Be more specific! Laser-assisted microdissection of plant cells. Trends Plant Sci. 10, 397-406. DOI: 10.1016/j.tplants.2005.06.006

Deng, Z., Cao, L., 2017: Fungal endophytes and their interactions with plants in phytoremediation: A review. Chemosphere 168, 1100-1106. DOI: 10.1016/j.chemosphere.2016.10.097

Dodds, P.N., 2010: Plant science: Genome evolution in plant pathogens. Science 330, 1486-1487. DOI: 10.1126/science.1200245

Eckardt, N.A., 2011: A symbiotic sugar transporter in the arbuscular mycorrhizal fungus *Glomus sp*. Plant Cell 23, 3561-3561. DOI: 10.1105/tpc.111.231010

Gaind, S., 2016: Phosphate dissolving fungi: Mechanism and application in alleviation of salt stress in wheat. Microbiol. Res. 193, 94-102. DOI: 10.1016/j.micres.2016.09.005

Ge, L., Sun, S., Chen, A., Kapulnik, Y., Xu, G., 2008: Tomato sugar transporter genes associated with mycorrhiza and phosphate. Plant Growth Reg. 55, 115-123. DOI: 10.1007/s10725-008-9266-7

Gutjahr, C., Parniske, M., 2013: Cell and developmental biology of arbuscular mycorrhiza symbiosis. Ann. Rev. Cell Dev. Biol. 29, 593-617. DOI: 10.1146/annurev-cellbio-101512-122413

Harrison, M.J., 1996: A sugar transporter from *Medicago truncatula*: altered expression pattern in roots during vesicular-arbuscular (VA) mycorrhizal associations. Plant J. 9, 491-503. DOI: 10.1046/j.1365-313X.1996.09040491.x

Henke, C., Jung, E.-M., Kothe, E., 2015: Hartig' net formation of *Tricholoma vaccinum*-spruce ectomycorrhiza in hydroponic cultures. Env. Sci. Poll. Res. 22, 19394-19399. DOI: 10.1007/s11356-015-4354-5

Humphreys, C.P., Franks, P.J., Rees, M., Bidartondo, M.I., Leake, J.R., Beerling, D.J., 2010: Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants. Nature Comm. 1, 103. DOI: 10.1038/ncomms1105

Kemen, A.C., Agler, M.T., Kemen, E., 2015: Host-microbe and microbe-microbe interactions in the evolution of obligate plant parasitism. New Phytol. 206, 1207-1228. DOI: 10.1111/nph.13284

Kothe, E., Turnau, K., 2018: Mycorrhizosphere communication: mycorrhizal fungi and endophytic fungus-plant interactions. Front. Microbiol. 9, 3015. DOI: 10.3389/fmicb.2018.03015

Lee, D.-K., Ahn, S., Cho, H.Y., Yun, H.Y., Park, J.H., Lim, J., Lee, J., Kwon, S.W., 2016: Metabolic response induced by parasitic plant-fungus interactions hinder amino sugar and nucleotide sugar metabolism in the host. Sci. Rep. 6, 37434. DOI: 10.1038/srep37434

Lei, J., Dexheimer, J., 1988: Ultrastructural localization of ATPase activity in the *Pinus sylvestris/Laccaria laccata* ectomycorrhizal association. New Phytol. 108, 329-334. DOI: 10.1111/j.1469-8137.1988.tb04170.x

Lundén, K., Danielsson, M., Durling, M.B., Ihrmark, K., Nemesio Gorriz, M., Stenlid, J., Asiegbu, F.O., Elfstrand, M., 2015: Transcriptional responses associated with virulence and defence in the interaction between *Heterobasidion annosum* s.s. and Norway spruce. PLoS One 10:e0131182. DOI: 10.1371/journal.pone.0131182

Martin, F.M., Uroz, S., Barker, D.G., 2017: Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria. Science 356, pii: eaad4501. DOI: 10.1126/science.aad4501

Mayer, A.M., 1989: Plant-fungal interactions: A plant physiologist's viewpoint. Phytochemistry 28, 311-317. DOI: 10.1016/0031-9422(89)80002-0

Mbengue, M., Navaud, O., Peyraud, R., Barascud, M., Badet, T., Vincent, R., Barbacci, A., Raffaele, S., 2016: Emerging trends in molecular interactions between plants and the broad host range fungal pathogens *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Front. Plant Sci. 7, 422. DOI: 10.3389/fpls.2016.00422

Moricca, S., Ragazzi, A., 2008: Fungal endophytes in mediterranean oak forests: A lesson from *Discula quercina*. Phytopathology 98, 380-386. DOI: 10.1094/PHYTO-98-4-0380

Parniske, M., 2008: Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nature Rev. Microbiol. 6, 763. DOI: 10.1038/nrmicro1987

Piqueras, J., 2001: Infection of *Trientalis europaea* by the systemic smut fungus *Urocystis trientalis*: disease incidence, transmission and effects

on performance of host ramets. J. Ecol. 87, 995-1004. DOI: 10.1046/j.1365-2745.1999.00409.x

PLETT, J.M., DAGUERRE, Y., WITTULSKY, S., VAYSSIÈRES, A., DEVEAU, A., MELTON, S.J., KOHLER, A., MORRELL-FALVEY, J.L., BRUN, A., VENEAULT-FOURREY, C., MARTIN, F., 2014: Effector MiSSP7 of the mutualistic fungus *Laccaria bicolor* stabilizes the *Populus* JAZ6 protein and represses jasmonic acid (JA) responsive genes. Proc. Natl. Acad. Sci. USA 111, 8299-8304. DOI: 10.1073/pnas.1322671111

RAI, M., AGARKAR, G., 2016: Plant-fungal interactions: What triggers the fungi to switch among lifestyles? Critical Rev. Microbiol. 42, 428-438. DOI: 10.3109/1040841X.2014.958052

SELIN, C., DE KIEVIT, T.R., BELMONTE, M.F., FERNANDO, W.G.D., 2016: Elucidating the role of effectors in plant-fungal interactions: progress and challenges. Front. Microbiol. 7, 600. DOI: 10.3389/fmicb.2016.00600

SHAW, R.G., ETTERSON, J.R., 2012: Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. New Phytol. 195, 752-765. DOI: 10.1111/j.1469-8137.2012.04230.x

SOYER, J.L., EL GHALID, M., GLASER, N., OLLIVIER, B., LINGLIN, J., GRANDAUBERT, J., BALESDENT, M.-H., CONNOLLY, L.R., FREITAG, M., ROUXEL, T., FUDAL, I., 2014: Epigenetic control of effector gene expression in the plant pathogenic fungus *Leptosphaeria maculans*. PLOS Genet. 10, e1004227. DOI: 10.1371/journal.pgen.1004227

STASKAWICZ, B.J., 2001: Genetics of plant-pathogen interactions specifying plant disease resistance. Plant Physiol. 125, 73-76. DOI: 10.1104/pp.125.1.73

TAERUM, S.J., DE BEER, Z.W., MARINCOWITZ, S., JANKOWIAK, R., WINGFIELD, M.J., 2018: *Ophiostoma quercus*: An unusually diverse and globally widespread tree-infecting fungus. Fungal Biol. 122, 900-910. DOI: 10.1016/j.funbio.2018.05.005

VANNIER, N., MONY, C., BITTEBIÈRE, A.-K., VANDENKOORNHUYSE, P., 2015: Epigenetic mechanisms and microbiota as a toolbox for plant phenotypic adjustment to environment. Front. Plant Sci. 6, 1159. DOI: 10.3389/fpls.2015.01159

VOSS, S., BETZ, R., HEIDT, S., CORRADI, N., REQUENA, N., 2018: RiCRN1, a crinkler effector from the arbuscular mycorrhizal fungus *Rhizophagus irregularis*, functions in arbuscule development. Front. Microbiol. 9, 2068. DOI: 10.3389/fmicb.2018.02068

WAGNER, K., LINDE, J., KRAUSE, K., GUBE, M., KOESTLER, T., SAMMER, D., KNIEMEYER, O., KOTHE, E., 2015: *Tricholoma vaccinum* host communication during ectomycorrhiza formation. FEMS Microbiol. Ecol. 91, fiv120. DOI: 10.1093/femsec/fiv120

ZEILINGER, S., GUPTA, V.K., DAHMS, T.E.S., SILVA, R.N., SINGH, H.B., UPADHYAY, R.S., GOMES, E.V., TSUI, C.K.-M., NAYAK, S.C., 2016: Friends or foes? Emerging insights from fungal interactions with plants. FEMS Microbiol. Rev. 40, 182-207. DOI: 10.1093/femsre/fuv045

ZHANG, L., SHI, N., FAN, J., WANG, F., GEORGE, T.S., FENG, G., 2018: Arbuscular mycorrhizal fungi stimulate organic phosphate mobilization associated with changing bacterial community structure under field conditions. Env. Microbiol. 20, 2639-2651. DOI: 10.1111/1462-2920.14289

ZHANG, L., WANG, M.-X., LI, H., YUAN, L., HUANG, J.-G., PENFOLD, C., 2014: Mobilization of inorganic phosphorus from soils by ectomycorrhizal fungi. Pedosphere 24, 683-689. DOI: 10.1016/S1002-0160(14)60054-0

ZHAO, J., SHAN, T., MOU, Y., ZHOU, L., 2011: Plant-derived bioactive compounds produced by endophytic fungi. Mini-Rev. Med. Chem. 11, 159-168. DOI: 10.2174/138955711794519492

ORCID
Erika Kothe (ID) https://orcid.org/0000-0002-8777-8004
Katrin Krause (ID) http://orcid.org/0000-0002-1336-8870

Address of the authors:
Friedrich Schiller Universityl Institute of Microbiology – Microbial Communication, Neugasse 25, 07743 Jena, Germany