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ORIGINAL ARTICLE

Hygrocybe virginea is a systemic endophyte of *Plantago lanceolata*

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Abstract Species of *Hygrocybe* (waxcaps) are mostly colorful mushrooms, which are characteristic of undisturbed grasslands. These fungi are endangered in many places worldwide, but their biology remains a mystery: while isotopic signatures indicate that waxcaps are neither mycorrhizal nor saprotrophic, they were recently observed in plant roots and molecularly detected in aboveground tissues. We aimed to establish a model system of *Plantago lanceolata* plants colonized by *H. coccinea* for future detailed studies of the plant–fungus association, and species-specific primers were designed to control infection success and screen environmental samples for waxcaps. The experimentally treated plants grown from surface-sterilized seeds were indeed colonized by waxcaps after 22 weeks of incubation. However, the fungal infection was independent from the experimental treatment and apparently resulted from infected seeds. Screening of field material confirmed that at least one species, i.e., *H. virginea*, is a maternally transmitted endophytic fungus associated with *P. lanceolata*. In the experiments, it obviously expanded to the roots during or after seed germination. The endophytic growth is also consistent with the carbon isotopic signature of *Hygrocybe*, which deviates less from the host plants' signature than known from ectomycorrhizal associations. However, waxcaps obviously acquire nitrogen (N) from a source outside the plant, like mycorrhizal fungi do. The extensive root system of *P. lanceolata* is hypothesized to facilitate reaching of nitrogen sources for *Hygrocybe* which are enriched in the heavier ^{15}N isotope.

Keywords Hygrocybe virginea · Hygrocybe coccinea · Plantago lanceolata · Endophytic · Basidiomycota · Isotopic signature

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Introduction

Species of the basidiomycetous genus *Hygrocybe* (waxcaps) are uncultivable Basidiomycota constituting a rather prominent component of undisturbed grasslands, the "waxcap grasslands" (Griffith et al. 2002). Due to the decline of their habitats, waxcaps are endangered in several regions of Australia (Kearney and Kearney 2007) and Europe (cf. www.wsl.ch/eccf/), at least. Their biology remains a mystery, and their mode of nutrition is currently considered to be "probably neither ECM [ectomycorrhizal] nor saprotrophic" (Seitzman et al. 2011). However, Halbwachs et al. (in press) recently observed hyphae of several *Hygrocybe* spp. within living plant roots, which suggests a mycorrhizal association. At the same time, Peršoh (2013) presented molecular evidence for the presence of *H. coccinea* in surface sterilized aboveground plant tissues. Accordingly, *Hygrocybe* spp. seem to exhibit traits of both, endophytic and mycorrhizal symbioses.

While both types of symbioses evolved multiple times independently in the Ascomycota and Basidiomycota, the diversity of mycorrhizal fungi is by far higher within the Basidiomycota and endophytic fungi mostly belong to the Ascomycota (Tedersoo et al. 2010; Unterseher 2011). Common features of mycorrhizal and most endophytic associations are that they are localized within or restricted to certain plant organs and only established after seed germination (Smith and Read 2008; Rodriguez et al. 2009). While

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symbioses with roots are easily established in soil, where both partners co-occur, the life cycles of endophytic fungi require reinfection of aboveground tissues by aerial spores or insect vectors for completion (Pažoutová et al. 2013). Vertically transmitted systemic infections are found among endophytic Ascomycota, with clavicipitacean endophytes of grasses being the most prominent one (Tanaka et al. 2012). The only further examples of non-pathogenic systemic infections also involve monocots, while their vertically transmitted (i.e. maternally inherited with the seeds) fungal symbionts belong to the Pleosporales (Redman et al. 2002; Ernst et al. 2003). Benefits for the host plants predominantly arise from nutrient supply from the surrounding soil in mycorrhizal symbioses (Read et al. 2004), while endophytic fungi may produce metabolites and enzymes increasing stress tolerance or herbivore resistance of the plant (Saikkonen et al. 2010; Debbab et al. 2012; Hamilton et al. 2012; Estrada et al. 2013). However, non-mycorrhizal root-endophytic fungi may be also involved in nutrient acquisition of the host plant (Usuki and Narisawa 2007; Behie et al. 2012). In contrast to mycorrhizal and root-endophytic fungi which permanently grow in association with living roots, most fungi within aboveground plant organs occur only temporarily as symptomless colonizers (Bills et al. 2012; Peršoh 2013). The transitional endophytic stage often precedes a saprotrophic or plant pathogenic state (Porras-Alfaro and Bayman 2011). However, the significance of the endophytic phase is still unknown for many common endophytes, such as the Xylariaceae, where host preference of the endophytic state is mostly inconsistent with that of the saprotrophic, stromata forming stage (Davis et al. 2003; Peršoh et al. 2010; Bills et al. 2012).

Accordingly, the presence of *Hygrocybe* hyphae in roots and aboveground tissues indicates a non-localized infection of the host plants, which is so far not known from non-pathogenic Basidiomycota. However, colonization of below and aboveground organs may also represent independent states in the life cycle of *Hygrocybe*, as suggested for Xylariaceae. Accordingly, we intended to infect *Plantago lanceolata* plants with *H. coccinea* to establish a model system for future detailed investigations of the plant-fungus association. This combination was chosen because *H. coccinea* was previously detected endophytically (Peršoh 2013), because we observed its fruiting bodies regularly in immediate proximity of *P. lanceolata* plants, and because *P. lanceolata* is the second commonest dicot species at the sampling sites (next to *Trifolium pratense*, which is associated with nitrogen-fixing bacteria).

Material and methods

In October 2011, seeds of *Plantago lanceolata* and fruiting bodies of *H. coccinea* were collected from a meadow in Bavaria, Germany (N 49° 38′ 43″, E 9° 12′ 4″). Co-occurring fruiting bodies of two further common waxcaps were collected in addition and later identified as *H. pratensis* var. *pallida, and H.* cf. *pratensis*. The ITS region of voucher specimens was sequenced and the vouchered collections were deposited in the collection of R. Agerer at the Botanische Staatssammlung München (M), under the accession numbers RA15041, RA15043, RA15277, and RA15278. Seeds collected from >15 *P. lanceolata* individuals were pooled and surface-sterilized by successive incubation under continuous agitation (120 rpm) in 70 % ethanol (1 min), 5 % Dinamin C + Tween (20 min) and sterile water (5 min). Sterilized seeds were incubated in Petri dishes with sterile sand for one month. Seedlings were transplanted into 150 ml pots with sterile sand. During this procedure, cavities prepared for planting the seedlings were treated with spore suspensions obtained from the collected fruiting bodies. In this way, each of 16 seedlings was inoculated with spores from one of the three collected *Hygrocybe* taxa [3 treatments (by *H. coccinea*, *H. pratensis* var. *pallida, and H.* cf. *pratensis*, respectively)×16 replicates=48 setups]. Plants were grown in the green house under plastic covers, to avoid cross-contamination between treatments. After 22 weeks, 6, 8, and 6 plant individuals inoculated with *H. coccinea*, *H. pratensis* var. *pallida, and H.* cf. *pratensis*, respectively, were selected for molecular analyses. From these plants, samples of 0.5 g fresh weight were dissected from random positions of leaves and roots under sterile conditions for DNA extraction.

In 2012, 18 individuals of *P. lanceolata* plants and >100 seeds from 16 plants were collected in the field and surface-sterilized following a protocol previously established to efficiently remove epiphytic fungi and adhering DNA (Peršoh et al. 2013), i.e., by treatment with 70 % ethanol (2 min), 1.2 % NaOCl (5 min), 70 % ethanol (1 min), and finally three rinses with sterile water (3×1 min). Seeds were thereby randomly selected and either pooled (10 samples of 2 seeds) or individually processed (11 samples of single seeds), and samples from plant organs were prepared as described above.

Species specific forward (Fw) and reversely (Rv) orientated primers, amplifying part of the ITS region by binding in the ITS1 and ITS2 region, respectively, were designed for *H. coccinea* (Fw: 5′-TGAGAAAGCAAACCTTGG-3′; Rv: 5′-GAATAAGAGAGCCTCTTCG-3′) using publicly available sequences. In the light of the first results (see below), additional primers were designed, which selectively amplified the taxa *H. pratensis* var. *pratensis* (Fw: 5′-CACTTYTTGTAG ATGCTGG-3′; Rv: 5′-CACACCAGAAACCAAGTC-3′) and *H. virginea* (Fw: 5′-CTTCCCTTGCTGTTTCTG-3′; Rv: 5′-GAAGCTGAAGTTCCCATTG-3′). These primers were applied to screen the samples for the two *Hygrocybe* taxa commonly encountered at the sampling site, but not involved in the experiments, because the detections of *H. coccinea* appeared not to be ascribable to the experimental treatment (i.e., inoculation).

Table 1 Detections of *Hygrocybe* spp. in *Plantago lanceolata*. The number of leaf, root and seed samples in which the fungi have been detected by species-specific PCR is given for each *Hygrocybe* spp. in the final two columns. Only species not inoculated to the respective plants were detected in the experiments (see Fig. 1). The second column notes the infection rates inferred from screening of the field material. Numerals in superscript indicate the number of detections verified by sequencing. The number of individuals (N_I) represented by the samples analysed (N_S) is given in parenthesis, followed by the number of positively tested individuals (P_I) and the number of independent PCR batches yielding positive results (P_B): N_S/ N_I // P_I / P_B. Verification of positive results in the same laboratory (^{R}) and at the University of Bayreuth (^{BT}) is indicated as well as detections achieved partly in both laboratories ($^{M+BT}$)

		Percent of infected plant individuals	Detections in field material	Detections in experimental treatments
H. virginea	Seed	5−29 %	6^2 (21/1−21//1−6/3^{M+BT})	n.a.
	Leaf	28 %	5 (18/18//4/2)	0 (12/12//0/0)
	Root	17 %	3^1 (18/18//3/2)	2^2 (20/20//2/$1^{R, BT}$)
H. coccinea	Seed	0 %	0 (21/1−21//0/0)	n.a.
	Leaf	0 %	0 (18/18//0/0)	1^1 (12/12//1/1^R)
	Root	6 %	1^1 (18/18//1/1)	2^2 (20/20//2/1^R)

Total DNA was extracted from all samples using the ChargeSwitch® gDNA Plant Kit (Invitrogen) following the manufacturer's instructions. Cell disruption was accomplished by running the FastPrep®-24 instrument (MP Biomedicals) at 6.5 ms^{-1} for 30 s, with 10 ceramic (Ø=1.4 mm) and 0.15 g of glass beads (Ø=0.5 mm) added to the samples as well as the nucleic acid extraction buffer supplied with the DNA extraction kit. PCR from plant samples was conducted using GoTaq® Flexi DNA Polymerase (Promega) as recommended by the manufacturer. Altogether, 17 independent batches of 4–34 single PCR reactions, respectively, were prepared in our laboratory in Munich throughout the study. Each batch was complemented by 1–2 negative controls. Screening of the DNA from inoculated plants for *H. virginea* was repeated in the Mycology Department of the University of Bayreuth using standard Taq DNA Polymerase (Invitrogen). Selected seed samples were also amplified there, to certainly avoid an incidental cross-contamination with *Hygrocybe* material, because neither specimens nor DNA of waxcaps have ever been handled in this laboratory. Cycling reactions started with an initial denaturation of 2 min at 95 °C followed by 37 cycles of 40 s at 94 °C, 1 min at 61 °C and 2 min at 72 °C. They finished with an extension step of 7 min at 72 °C. Affiliation of amplicons to species was verified by sequencing, conducted by the Sequencing Service of the LMU (Munich).

Results and discussion

Hygrocybe coccinea was not detected in the six randomly selected *P. lanceolata* individuals inoculated with the fungus' spores (Table 1), indicating that the experiments to establish a reproducible model system failed. However, DNA of *H. coccinea* was found in roots and leaves, respectively, of two out of 8 tested plants inoculated with *H. pratensis* var. *pallida* and in the roots of one out of six analysed plants inoculated with *H.* cf. *pratensis* (Fig. 1). Based on these results, the hypothesis emerged that *H. coccinea* was already present in the seeds used to grow the plants. Therefore, we screened for additional *Hygrocybe* taxa (i.e., *H. pratensis* var. *pratensis* and *H. virginea*), which were not included in the experiments, but common at the sampling sites. *Hygrocybe pratensis* var. *pratensis* was not detected, but *H. virginea* was detected in the roots of two *P. lanceolata* individuals inoculated with *H.* cf. *pratensis*. The identities of amplicon sequences of *H. virginea* were verified by sequence analyses. Furthermore, re-analysis of 15 randomly selected plant samples in a definitely "waxcap-free laboratory" yielded identical results with a

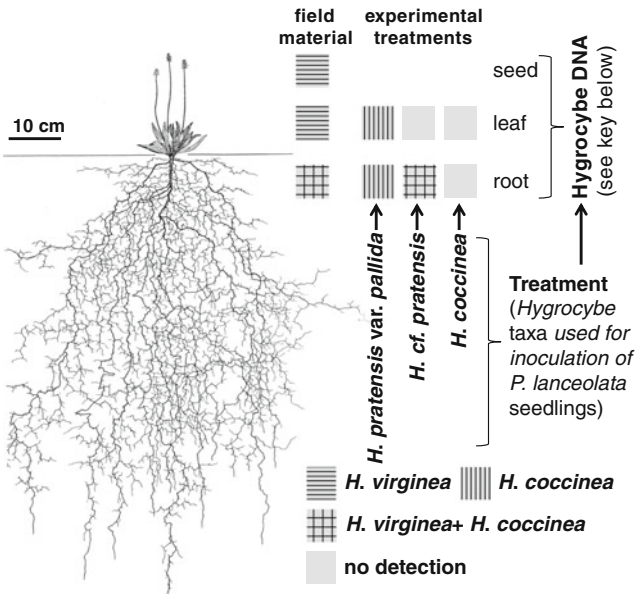

Fig. 1 Detection of *Hygrocybe* spp. (waxcaps) in organs and seeds of *Plantago lanceolata*. *Hygrocybe coccinia* was detected after 22 weeks in plants grown under sterile conditions, but only in plants treated with other species of *Hygrocybe* (three columns on the right). *Hygrocybe virginea* was detected, even though it was not inoculated. The presence of *H. viginea* in seeds of field material (left column) also indicates vertical transmission of the fungus. Habitus drawing of *P. lanceolata* reproduced from Kutschera and Lichtenegger (1992), with permission

different PCR Kit, i.e. the same 13 negative and 2 positive results. Accordingly, the endophytic occurrence of *H. coccinea* and *H. virginea* in *P. lanceolata* was independent from the experimental treatment. This was the first evidence that *Hygrocybe* spp. may be systemic endophytes transmitted with the host plant seeds. In the plants grown under sterile conditions, the fungi obviously invaded, starting from the infected seed, roots and leaves during or after seed germination.

Subsequent analyses of freshly collected and surface-sterilized *P. lanceolata* individuals strengthened the hypothesis of an endophytic growth of waxcaps: While there was no evidence of *H. pratensis* var. *pratensis* colonizing *P. lanceolata*, *H. coccinea* was detected once in roots and *H. virginea* five and three times in leaves and roots, respectively (Fig. 1, Table 1). Furthermore, *H. virginea* occurred in six out of 21 analyzed surface-sterilized seeds, which represented a random selection from >100 seeds collected from 16 plant individuals. This second evidence for a vertical transmission of *H. virginea* with the plant seeds was also produced in two laboratories and confirmed by sequence analysis. Between 5 % and 29 % of the host individuals carried infected seeds, and infection rates of the vegetative organs were also in this range (Table1). Even though these are only preliminary estimates, to be confirmed by larger scaled sampling approaches, they already indicate that *P. lanceolata* is regularly but not predominantly associated with *H. virginea* at the sampling site. The association between *H. coccinea* and *P. lanceolata* was rarer (Table 1), and may be less intimate. Even though vertical transmission of this species with host seeds is the most plausible explanation for its occurrence in the experimental plants, we were not able to obtain direct evidence for its presence in seeds of *P. lanceolata*. Moreover, *H. coccinea* was also found to colonize mistletoes and exceptionally pines endophytically (Peršoh 2013), indicating a broad host range for the species. However, while we commonly observed fruiting bodies of *H. coccinea* and *H. virginea* in close proximity to *P. lanceolata*, both also occur close to other plants, such as *Hypochaeris radicata* and *Leontodon hispidus*, at the sampling site. This may indicate a rather broad host range for both species, which certainly has to be analyzed in more detail in future studies. Still, this study provides for the first time evidence for a systemic infection of a dicot plant by a non-pathogenic basidiomycote and for its maternal inheritance: *H. virginea* is a vertically transmitted systemic endophyte of *P. lanceolata*.

In this context, it should be noted that *H. virginea* and *H. coccinea* are phylogenetically not as closely related as implied by their current assignment to the same genus. While we used the accepted and most commonly applied names in this study, there is strong evidence that *H. virginea* (as well as *H. pratensis*) will have to be transferred to the genus *Cuphophyllus* (Babos et al. 2011). Accordingly, the species analyzed here represent the most distantly related lineages within the Hygrophoraceae (Lodge et al. 2006).

Their endophytic occurrence may also be the key towards understanding the currently mysterious biology of waxcaps. The mystery of waxcap biology predominantly concerns their unusual enrichment in ^{15}N and ^{13}C isotopes (Griffith et al. 2002; Seitzman et al. 2011), as compared to their probable carbon source, i.e., their host plants or plant substrates (Kohzu et al. 1999). Such an isotopic signature suggests a mutualistic lifestyle as exhibited by mycorrhizal fungi, i.e. indicating nitrogen supply for and carbon supply from the host plant (Kohzu et al. 1999; Hobbie and Högberg 2012). However, compared to ectomycorrhizal (ECM) fungal fruit bodies (Mayor et al. 2009), ^{15}N-values of *Hygrocybe* spp. are rather high, while ^{13}C-values range between plants and ECM fungi. Mycorrhizal fungi obtain sucrose from the host plant, which is richer in ^{13}C than the plant on average (Gleixner et al. 1993). As systemic endophytes, *Hygrocybe* spp. possibly utilize plant compounds with lower ^{13}C content than sucrose, such as monosaccharides (Gleixner et al. 1993), in addition or alternatively to sucrose. This would explain the lower ^{13}C enrichment observed in *Hygrocybe spp*., as compared to ECM fungi. The nitrogen source of *Hygrocybe*, again, is obviously enriched in ^{15}N, which applies to mycorrhizal fungi (l.c.), invertebrates (Oelbermann and Scheu 2010), and highly decomposed organic material (Gebauer and Taylor 1999; Hobbie and Högberg 2012). While reports on mycorrhizal fungi serving as nitrogen source for other plant-associated fungi are lacking so far, direct nitrogen transfer from arthropods to plants via an entomopathogenic and root-endophytic fungus has recently been presented (Behie et al. 2012). Even though the finding that *Hygrocybe* colonizes plants internally provides no clues about its obviously external nitrogen source, highly branched and deep-reaching root systems of host plants such as *P. lanceolata* (Fig. 1; Kutschera and Lichtenegger 1992) may facilitate reaching sources such as ^{15}N-enriched organic material in lower soil horizons.

Due to the reluctance of *Hygrocybe* spp. to grow in culture, final assessment of the significance of the endophytic occurrence for the life cycle of *Hygrocybe* will be a major challenge. This will probably require analyses of environmental meta-transcriptomes against the background of fruit body-derived reference genomes.

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References

Babos M, Halász K, Zagyva T, Zöld-Balogh Á, Szegő D, Bratek Z (2011) Preliminary notes on dual relevance of ITS sequences and pigments in *Hygrocybe* taxonomy. Persoonia 26:99–107

Behie SW, Zelisko PM, Bidochka MJ (2012) Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. Science 336:1576–1577

Bills GF, Gonzalez-Menendez V, Martin J, Platas G, Fournier J, Peršoh D, Stadler M (2012) *Hypoxylon pulicicidum* sp nov (Ascomycota, Xylariales), a pantropical insecticide-producing endophyte. Plos One 7:e46687

Davis EC, Franklin JB, Shaw AJ, Vilgalys R (2003) Endophytic *Xylaria* (Xylariaceae) among liverworts and angiosperms: phylogenetics, distribution, and symbiosis. Am J Bot 90:1661–1667

Debbab A, Aly A, Proksch P (2012) Endophytes and associated marine derived fungi—ecological and chemical perspectives. Fungal Divers 57:45–83

Ernst M, Mendgen KW, Wirsel SGR (2003) Endophytic fungal mutualists: seed-borne *Stagonospora* spp. enhance reed biomass production in axenic microcosms. Mol Plant-Microbe Interact 16:580–587

Estrada C, Wcislo WT, Van Bael SA (2013) Symbiotic fungi alter plant chemistry that discourages leaf-cutting ants. New Phytol 198:241–251

Gebauer G, Taylor AFS (1999) ^{15}N natural abundance in fruit bodies of different functional groups of fungi in relation to substrate utilization. New Phytol 142:93–101

Gleixner G, Danier HJ, Werner RA, Schmidt HL (1993) Correlations between the C-13 content of primary and secondary plant-products in different cell compartments and that in decomposing basidiomycetes. Plant Physiol 102:1287–1290

Griffith GW, Easton GL, Jones AW (2002) Ecology and diversity of waxcap (*Hygrocybe* spp.) Fungi. Bot J Scotl 54:7–22

Halbwachs H, Dentinger BT, Detheridge AP, Karasch P, Griffith GW (in press) Hyphae of waxcap fungi colonise plant roots. Fungal Ecology

Hamilton C, Gundel PE, Helander M, Saikkonen K (2012) Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. Fungal Divers 54:1–10

Hobbie EA, Högberg P (2012) Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. New Phytol 196:367–382

Kearney R, Kearney E (2007) The listing of an Australian Hygrocybeae community: and its holotype species under State and Commonwealth legislations. Field Mycology 8:13–21

Kohzu A, Yoshioka T, Ando T, Takahashi M, Koba K, Wada E (1999) Natural 13C and 15N abundance of field-collected fungi and their ecological implications. New Phytol 144:323–330

Kutschera L, Lichtenegger E (1992) Wurzelatlas mitteleuropäischer Grünlandpflanzen. Gustav Fischer Verlag, Stuttgart, Jena, New York

Lodge DJ, Matheny PB, Cantrell SA, Moncalvo J-M, Vilgalys R, Redhead S (2006) Delineating the Hygrophoraceae: character myths vs. gene trees. Inoculum 57:27. Poster available at http://www.aber.ac.uk/waxcap/downloads/Lodge2006-HygrophoraceaeMSAposter.pdf

Mayor JR, Schuur EAG, Henkel TW (2009) Elucidating the nutritional dynamics of fungi using stable isotopes. Ecol Lett 12:171–183

Oelbermann K, Scheu S (2010) Trophic guilds of generalist feeders in soil animal communities as indicated by stable isotope analysis (15N/14N). Bull Entomol Res 100:511–520

Pažoutová S, Follert S, Bitzer J, Keck M, Surup F, Šrůtka P, Holuša J, Stadler M (2013) A new endophytic insect-associated *Daldinia* species, recognised from a comparison of secondary metabolite profiles and molecular phylogeny. Fungal Divers. doi:10.1007/s13225-013-0238-5

Peršoh D (2013) Factors shaping community structure of endophytic fungi–evidence from the *Pinus*-*Viscum*-system. Fungal Divers 60:55–69

Peršoh D, Melcher M, Flessa F, Rambold G (2010) First fungal community analyses of endophytic ascomycetes associated with *Viscum album* ssp. *austriacum* and its host *Pinus sylvestris*. Fungal Biol 114:585–596

Peršoh D, Segert J, Zigan A, Rambold G (2013) Fungal community composition shifts along a leaf degradation gradient in a European beech forest. Plant Soil 362:175–186

Porras-Alfaro A, Bayman P (2011) Hidden Fungi, Emergent Properties: Endophytes and Microbiomes. Annu Rev Phytopathol 49:291–315

Read DJ, Leake JR, Perez-Moreno J (2004) Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Can J Bot 82:1243–1263

Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002) Thermotolerance Generated by Plant/Fungal Symbiosis. Science 298:1581

Rodriguez RJ, White JF, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314–330

Saikkonen K, Saari S, Helander M (2010) Defensive mutualism between plants and endophytic fungi? Fungal Divers 41:101–113

Seitzman BH, Ouimette A, Mixon RL, Hobbie EA, Hibbett DS (2011) Conservation of biotrophy in Hygrophoraceae inferred from combined stable isotope and phylogenetic analyses. Mycologia 103:280–290

Smith S, Read D (2008) Mycorrhizal Symbiosis. Academic Press and Elsevier, London

Tanaka A, Takemoto D, Chujo T, Scott B (2012) Fungal endophytes of grasses. Curr Opin Plant Biol 15:462–468

Tedersoo L, May T, Smith M (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263

Unterseher M (2011) Diversity of Fungal Endophytes in Temperate Forest Trees. In: Pirttilä AM, Frank AC (eds) Endophytes of forest trees. Springer, Netherlands, pp 31–46

Usuki F, Narisawa K (2007) A mutualistic symbiosis between a dark septate endophytic fungus, *Heteroconium chaetospira*, and a nonmycorrhizal plant, Chinese cabbage. Mycologia 99:175–184