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Recent developments in the study of orchid mycorrhiza

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Abstract

Orchids are mycoheterotrophic during their seedling stage and in many species the dependency on fungi as a carbohydrate source is prolonged into adulthood. The mycobionts in orchid mycorrhiza belong in at least 5 major taxonomic groups of basidiomycetes. Traditional records have mainly focused on saprotrophic mycobionts but the participation of both ectomycorrhizal and parasitic fungi in orchid mycorrhiza has been corroborated. There is an increasing evidence of specific relationships between orchids and fungi, though usually not on a species-to-species level. Physiological compatibility demonstrated under artificial conditions, as *in vitro*, may be much broader, however. Recent development of field sowing techniques has improved the possibilities of evaluating orchid-fungal relations in an ecological context. Although the general nutrient flow in orchid mycorrhiza is well known, some questions remain regarding breakdown processes of fungi within orchid tissues, especially the ptyophagic syndrome that has recently been illustrated at the ultrastructural level for the first time.

Energy sources for orchid mycorrhiza in the field

Fungi associated with orchid mycorrhiza (OM) have traditionally been mostly regarded as saprotrophs, dead organic material thus being the energy source for the symbiosis. This is supported by well documented cases where orchids have developed with fungi cultured on organic additives *in vitro*, or on organic debris. A water agar with the addition of ground woodchips can sustain some orchid-fungus symbioses from seed to small plant (Whigham et al., MS). Many of the orchid endophytes that are referred to *Rhizoctonia* DC (*sensu lato*) are saprophytes (Roberts, 1999). *Thanatephorus ochraceus* (Massee)P.Roberts (Table 1) for example, grows on decaying wood and dead fern fronds (Roberts, 1998). Rhizoctonias associated with orchids produce a range of carbohydrate-degrading and other enzymes enabling the breakdown of plant debris (a summary in Rasmussen, 1995). *Rhizoctonia* strains referred to *Ceratorhiza* R.T. Moore produce polyphenoloxidases, which are active in the breakdown of lignin (Zelmer et al., 1996). Species of *Mycena* that associate with species of *Cymbidium* and *Gastrodia* (Fan et al., 1996; Lan et al., 1996), are acknowledged saprotrophs. *Lentinus edodes* Berk., the shiitake mushroom, that is a white-rot saprotroph, can support the development of a chlorophyll-deficient orchid, *Erythrorchis ochobiensis* (Hayata) Garay, as can a range of other wood rotting fungi (Umata, 1998a). The natural symbiont of *E. ochobiensis*, as well as *Galeola altissima* (Bl.) Bl., another chlorophyll-deficient liana, is *Erythromyces crocicreas* (Berk.&Br.) Hjortst. & Ryv., the causal agent for white pocket rot in wood (Hjortstam and Tellería, 1990; Umata, 1995).

Some anastomosis groups of *Rhizoctonia solani* Kühn, a well known plant pathogen, are found in constant association with orchids (Carling et al., 1999; Perkins and McGee, 1995); similar associations have been described previously but usually as unstable relationships (survey in Rasmussen, 1995). The pathogenic rhizoctonias are generally necrotrophic parasites, first killing their host and subsequently living =saprotrophically from it (Roberts, 1999). This life form thus requires a considerable saprotrophic capacity (Garrett, 1962). Hypovirulent strains of *R. solani* exist and it is not known whether the energy

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Table 1. The diversity of fungi in mycorrhizal relationship with orchids involve saprotrophs, ectomyccorhizal and parasitic fungi and 5 major basidiomycete groups: **Heterobasidiomycetes**: *Ceratobasidium, Oliveonia, Thanatephorus, Tulasnella, Sebacina*, **'Hericianae'**: *Russula* , **'Hymenocaetanae'**: *Erythromyces*, **'Thelephoranae'**: *Thelephora-Tomentella*, and '**Agaricanae**': *Armillaria, Mycena* (higher basidiomycete; systematics according to informal system by Henrik Petersen, http://www.mycokey.com/aau/systematics/systematicsbasifuturistic.htm1.). Species combinations from well documented symbioses are listed, but this is not meant to imply that these species are exclusive to other symbionts. The list is not intended to be exhaustive but mainly comprises recent developments. ∗ Source not seen. ∗[2]Not evident from reference; found repeatedly in tissues of C.s. but germination test failed; yet germination test succeeded with *Gastrodia elata* and *Dendrobium* spp. ∗[3]According to Henrik Petersen *Oliveonia* belongs in Ceratobasidiales. ∗[4] Source not seen.

Teleomorph fungus	Anamorph fungus	Species of orchid	Synomyms of fungus	Fungal group	Orchid lifeform	Fungal lifeform	Source to fungal identity and synonymy	Source to relationship
Armillaria jezoensis Cha and Igarashi		*Galeola septentrionalis* Reichb.f.		Tricholomatales	terrestrial, chlorophyll deficient	parasite		Cha and Igarishi, 1996
Armillaria mellea (Vahl.Fr.) Karst.		*Gastrodia elata* Bl.		Tricholomatales	terrestrial, chlorophyll deficient, climber	parasite		Lan et al., 1994
Ceratobasidium bicorne J. Eriksss. and Ryvarden		*Prasophyllum macrostachyum* R. Br.	*Ypsilonidium anomalum* Warcup and Talbot =*Thanatephorus anomala*	Ceratobasidiales	terrestrial geophyte	saprotroph, parasite	Roberts, 1999	Warcup and Talbot, 1980
Ceratobasidium cornigerum (Bourdot) Rogers		*Acianthus reniformis* (R.Br.) Schltr.	*C. obscurum* Aut.	Ceratobasidiales	terrestrial geophyte	saprotroph, parasite	Roberts, 1999	Warcup and Talbot, 1971
Ceratobasidium cornigerum (Bourdot) Rogers		Canadian orchids	*C. obscurum* Aut.	Ceratobasidiales		saprotroph, parasite	Roberts, 1999	Currah et al, 1987
Ceratobasidium cornigerum (Bourdot) Rogers		*Goodyera repens* (L.) R.Br.		Ceratobasidiales	terrestrial rhizomatous evergreen	saprotroph, parasite	Roberts,1999	
Ceratobasidium cornigerum (Bourdot) Rogers	*Ceratorhiza goodyerae -repentis* (Costantin & Dufour) Moore	*Platanthera obtusata* (Banks ex Pursh) Lindl.		Ceratobasidiales	terrestrial geophyte	saprotroph	Roberts, 1999	Currah et al., 1990
Ceratobasidium cornigerum (Bourdot) Rogers		*Pterostylis* sp., *Prasophyllum* sp.		Ceratobasidiales	terrestrial geophyte	saprotroph, parasite	Roberts, 1999	
Ceratobasidium cornigerum (Bourdot) Rogers		*Sarcochilus* sp.	*C. papillatum* Warcup & Talbot	Ceratobasidiales	epiphytic, monopodial	saprotroph, parasite	Roberts, 1999	
Ceratobasidium cornigerum (Bourdot) Rogers		*Thrixspermum congestum* (F. M. Bail) Dockr.	*C. papillatum* Warcup & Talbot	Ceratobasidiales	epiphytic, monopodial	saprotroph, parasite	Roberts, 1999	
Ceratobasidium globisporum Warcup & Talbot		*Calanthe triplicata* (Willem.) Ames		Ceratobasidiales	terrestrial, pseudobulbous	only known as orchid symbiont	Roberts, 1999	
Ceratobasidium globisporum Warcup & Talbot		*Trichoglottis australiensis* Dockr.		Ceratobasidiales	epiphytic, monopodial	only known as orchid symbiont	Roberts, 1999	
Ceratobasidium pseudocornigerum M.P.Christ.		*Pterostylis mutica* R.Br. Warcup & Talbot	*C. angustisporum*	Ceratobasidiales	terrestrial geophyte	saprotroph	Roberts, 1999	Warcup & Talbot, 1980
Ceratobasidium sp.		*Taeniophyllum obtusum* Bl.		Ceratobasidiales	epiphytic, leafless, photosynthetic roots	saprotroph		Irawati, 1993*
Ceratobasidium sphaerosporum Warcup & Talbot		members of Sarcanthinae		Ceratobasidiales	epiphytic, monopodial	only known as orchid symbiont	Roberts, 2000	
Ceratobasidium sphaerosporum Warcup & Talbot		*Pomatocalpa macphersonii* (F. Muell.) Hunt		Ceratobasidiales	epiphytic, monopodial	only known as orchid symbiont	Roberts, 1999	Warcup & Talbot, 1971
Ceratobasidium sphaerosporum Warcup & Talbot		*Robiquetia wassellii* Dockrill		Ceratobasidiales	epiphytic, monopodial	only known as orchid symbiont	Roberts, 1999	Warcup & Talbot, 1971

Table 1. Continued

Erythromyces crocicreas (Berk. & Br.) Hjortst.&Ryv.	*Galeola altissima* (Bl.) Bl.	*Hymenochaete crocicreas* Berk. & Br.	Aphyllophorales	terrestrial, chlorophyll deficient, climber	saprotroph		Umata, 1995
Erythromyces crocicreas (Berk. & Br.) Hjortst.& Ryv.	*Erythrorchis ochobiense* (Hayata) Garay	*Hymenochaete crocicreas* Berk. & Br.	Hymenochaetales	terrestrial, chlorophyll deficient, climber	saprotroph		Umata, 1998
Moniliopsis anomala Currah	*Coeloglossum viride* (L.) Hartm.		Ceratobasidiales	terrestrial geophyte	only known as orchid symbiont		Currah et al., 1990
Moniliopsis anomala Currah	*Platanthera hyperborea* (L.) Lindl.		Ceratobasidiales	terrestrial geophyte	only known as orchid symbiont		Currah et al., 1990
Mycena orchidicola Fan et Guo	*Cymbidium sinense* (Andr.) Willd.		Tricholomatales	terrestrial rhizomatous	saprotroph		Fan et al., 1996*[2]
Mycena osmundicola Lange	*Gastrodia elata* Bl.		Tricholomatales	terrestrial, chlorophyll deficient, climber	saprotroph		Lan et al., 1996
Oliveonia pauxilla FK(H.S.Jacks.) Donk	?		Exidiales*[3]		saprotroph	Roberts, 1999	Warcup, 1975
Russulaceae spp.	*Corallorhiza maculata* (Rafin.) Rafin.		"Gloecystidiales"	terrestrial, chlorophyll deficient	ectomycorrhizal		Taylor and Bruns, 1999
Russulaceae spp.	*Corallorhiza mertensiana* Bongard		"Gloecystidiales"	terrestrial, chlorophyll deficient	ectomycorrhizal		Taylor and Bruns, 1999
Serendipita vermifera (Oberw.) P. Roberts	*Acianthus* spp.	*Sebacina vermifera* Oberw.	Exidiales	terrestrial geophyte	saprotroph, ectomycorrhizal	Roberts, 1999	
Serendipita vermifera (Oberw.) P. Roberts	*Caladenia* spp.	*Sebacina vermifera* Oberw.	Exidiales	terrestrial geophyte	saprotroph, ectomycorrhizal	Roberts, 1999	
Serendipita vermifera (Oberw.) P. Roberts	*Elythranthera* spp.	*Sebacina vermifera* Oberw.	Exidiales	terrestrial geophyte	saprotroph, ectomycorrhizal	Roberts, 1999	
Serendipita vermifera (Oberw.) P. Roberts	*Eriochilus* spp.	*Sebacina vermifera* Oberw.	Exidiales	terrestrial geophyte	saprotroph, ectomycorrhizal	Roberts, 1999	
Serendipita vermifera (Oberw.) P. Roberts	*Glossodia major* R.Br.	*Sebacina vermifera* Oberw.	Exidiales	terrestrial geophyte	saprotroph, ectomycorrhizal	Roberts, 1999	
Serendipita vermifera (Oberw.) P. Roberts	*Microtis* spp.	*Sebacina vermifera* Oberw.	Exidiales	terrestrial geophyte	saprotroph, ectomycorrhizal	Roberts, 1999	
Serendipita vermifera (Oberw.) P. Roberts	*Prasophyllum* spp.	*Sebacina vermifera* Oberw.	Exidiales	terrestrial geophyte	saprotroph, ectomycorrhizal	Roberts, 1999	
Sistotrema sp.	*Piperia unalascensis* (Spreng.) Rydb.			terrestrial geophyte	saprotroph		Currah et al., 1990
Sistotrema sp.	*Platanhera obtusata*(Banks ex Pursh) Lindl.			terrestrial geophyte	saprotroph		Currah et al., 1990
Thanatephorus cucumeris (A.B.Frank) Donk	*Prasophyllum odoratum* Rogers		Ceratobasidiales	terrestrial geophyte	parasite	Roberts, 1999	
Thanatephorus cucumeris (A.B.Frank) Donk	*Pterostylis foliata* Hook.f.		Ceratobasidiales	terrestrial geophyte	parasite	Roberts, 1999	
Thanatephorus gardneri Warcup nom dub.	*Rhizanthella gardneri* Rogers		Ceratobasidiales	subterranean, chlorophyll-deficient	orchid symbiont, ectomycorrhizal		Warcup, 1991
Thanatephorus ochraceus (Massee) P. Roberts	*Calypso bulbosa* (L) Oakes	*T. orchidicola* Warcup & Talbot, *T. pennatus* Currah	Ceratobasidiales	terrestrial geophyte	saprotroph	Roberts, 1998	Currah, 1987
Thanatephorus ochraceus (Massee) P.Roberts	*Coeloglossum viride* (L.) Hartm.	*T. orchidicola* Warcup & Talbot, *T. pennatus* Currah	Ceratobasidiales	terrestrial geophyte	saprotroph	Roberts, 1998	Warcup & Talbot 1967

Table 1. Continued

Teleomorph fungus	Anamorph fungus	Species of orchid	Synomyms of fungus	Fungal group	Orchid lifeform	Fungal lifeform	Source to fungal identity and synonymy	Source to relationship
Thanatephorus ochraceus (Massee) P. Roberts		*Orchis mascula* (L.) L.	*T. orchidicola* Warcup & Talbot, *T. pennatus* Currah	Ceratobasidiales	terrestrial geophyte	saprotroph	Roberts, 1998	Warcup & Talbot 1967
Thanatephorus sp		*Acampe praemorsa* (Roxb.) Blatter & McCann		Ceratobasidiales	epiphytic, monopodial	parasite or saprotroph		Senthilkumar, Vengadeswari and Krishnamurthy 2000
Thanatephorus sterigmaticus (Bourdot) Talbot		*Thelymitra antennifera* Hook.f.		Ceratobasidiales	terrestrial geophyte	saprotroph	Roberts, 1999	Warcup & Talbot 1967
Thelephoraceae sp.		*Cephalanthera austinae*(A.Gray) Heller		Thelephorales	terrestrial, chlorophyll deficient	ectomycorrhizal		Taylor & Bruns, 1997
Tulasnella cruciata Warcup & Talbot		*Acianthus caudatus* R. Br.		Tulasnellales	terrestrial geophyte	only known as orchid symbiont	Roberts, 1999	Warcup & Talbot, 1971
Tulasnella cruciata Warcup & Talbot		*Thelymitra* spp.		Tulasnellales	terrestrial geophyte	only known as orchid symbiont	Roberts, 1999	Warcup & Talbot, 1971
Tulasnella deliquescens (Juel) Juel	*Epulorhiza repens* (N. Bernard) R.T. Moore	*Dactylorhiza purpurella* (T & T.A. Stephenson) Soo	*T. calospora* aut.	Tulasnellales	terrestrial geophyte	saprophyte	Roberts, 1999	
Tulasnella deliquescens (Juel) Juel	*Epulorhiza repens* (N. Bernard) R.T. Moore	*Dendrobium* sp.	*T. calospora* aut.	Tulasnellales	epiphyte pseudobulbous	saprophyte	Roberts, 1999	
Tulasnella deliquescens (Juel) Juel	*Epulorhiza repens* (N. Bernard) R.T. Moore	*Diuris, Acianthus, Caladenia, Thelymitra, Lyperanthes* spp.	*T. calospora* aut.	Tulasnellales	terrestrial geophyte	saprophyte	Roberts, 1999	
Tulasnella irregularis Warcup & Talbot		*Dendrobium dicuphum* F. Muell.		Tulasnellales	epiphyte pseudobulbous	only known as orchid symbiont	Roberts, 1999	Warcup & Talbot 1980
Tulasnella pinicola Bres.		*Dendrobium* sp.	*T. assymmetrica* Warcup & Talbot	Tulasnellales	epiphyte pseudobulbous	saprotroph	Roberts, 1999 1967 eller 1972	Warcup & Talbot,
Tulasnella pinicola Bres.		*Thelymitra luteocilium* Fitzg.	*T. assymmetrica* Warcup & Talbot	Tulasnellales	terrestrial geophyte	saprotroph	Roberts, 1999 1967 eller 1971	Warcup & Talbot,
Tulasnella violea (Quél.) Bourd. & Galz.		*Thelymitra aristata* Lindl.		Tulasnellales	terrestrial geophyte	saprotroph	Roberts, 1999	Warcup & Talbot 1971
	Rhizoctonia repens N. Bernard	*Cymbidium goeringii* Reichb.f.		Fungi imperfecti	terrestrial rhizomatous	saprophyte		Lee et al., 1998*[4]
	Epulorhiza calendulina Zelmer & Currah	*Amerorchis rotundifolia* (Banks) Hulten		Fungi imperfecti	terrestrial geophyte	only known as orchid symbiont		Zelmer and Currah, 1995b
	Epulorhiza anaticula(Currah) Currah	*Coeloglossum viride* (L.) Hartm.		Fungi imperfecti	terrestrial geophyte	only known as orchid symbiont		Currah et al., 1990
	Epulorhiza repens (N. Bernard) R.T. Moore	*Microtis parviflora R. Br.*		Fungi imperfecti	terrestrial geophyte	saprophytes		Perkins et al, 1995
	Epulorhiza anaticula(Currah) Currah	*Plantanthera obtusata*(Banks ex Pursh) Lindl.		Fungi imperfecti	terrestrial geophyte	only known as orchid symbiont		Currah et al., 1990
	Epulorhiza anaticula(Currah) Currah	*Platanthera hyperborea* (L.) Lindl.		Fungi imperfecti	terrestrial geophyte	only known as orchid symbiont		Currah et al., 1990
	Ceratorhiza pernacatena Zelmer & Currah	*Platanthera praeclara* Sheviak & Bowles		Fungi imperfecti	terrestrial geophyte	only known as orchid symbiont		Zelmer & Currah, 1995b

source in OMs involving pathogenic Rhizoctonias is ever a living organism. Sen et al. (1999) assumed that the *Ceratorhiza* spp. they consistently found in roots of *Goodyera repens* (L.) R.Br. lived in a mildly pathogenic association with *Pinus silvestris* L. The main substrate of *Ceratorhiza* sp. in association with Scottish *G. repens* was assumed to be pine needle litter (Downie, 1943), but parasitic strains of *Ceratobasidium cornigerum* (Bourdot) Rogers (usually the teleomorph form of *G. repens* endophytes) exist on woody and herbaceous plants (Roberts, 1998). The mycobionts in certain species of *Galeola* (Cha et al., 1996; Terashita, 1996) belong to *Armillaria*, a genus of tree parasites.

Recent findings demonstrate that ectomycorrhizal fungi (ECM) may also participate in OM which means that living trees provide photosynthates in a triple symbiosis. This type of relationship has been suspected to exist in species of *Corallorhiza* since Campbell (1970) observed rhizomorphs adjacent to the rhizomes of *C. striata* Lindl. Recently, the relationship has been studied in considerable detail. Zelmer and Currah (1995a) isolated a clamp-bearing basio- diomycete from *C. trifida* Chatelain and verified that it could form ECM with seedlings of *Pinus contorta in vitro*. In populations of *C. maculata* (Rafin.) Rafin., Taylor and Bruns (1997) found endophytes which, by DNA-analyses, were identified to Russulaceae, again strongly suggesting an ECM relationship as the carbohydrate source. Most recently, McKendrick et al. (2000a) obtained isolates from field sown seedlings of *C. trifida* and by DNA sequencing identified them to the *Thelephora-Tomentella* complex of Thelephoraceae. In microcosms, they subsequently linked the OM to roots of *Betula pendula* and *Salix repens* in an ECM relationship, and by isotope tracing demonstrated the transfer of carbon from the tree through the fungal partner to the orchid (McKendrick et al., 2000b). This would seem to settle the case, as far as *Corallorhiza* is concerned. Since a small and short-lived inflorescense with little chlorophyll is the only aboveground structure produced in species of *Corallorhiza*, the contribution from the tree probably is crucial to the survival of the orchid. The drain on the trees, on the other hand, of supporting the orchids was estimated to be very modest (McKendrick et al., 2000b). Another case where an ectomycorrhizal tree could be involved as carbohydrate source is the chlorophyll-deficient orchid *Cephalanthera austinae* (A.Gray) Heller whose mycobionts belong to Thelephoraceae and showed similarity to fungi forming ECM on adjacent tree roots (Taylor and Bruns, 1997).

The nutritional basis of OM relationships may be further complicated when more than one endophyte form pelotons at the same time in the same orchid tissue, such as found in both chlorophyll-deficient and photosynthetic orchids (Scrugli and Cogoni, 1994; Zelmer et al., 1996), because these fungi could have different energy sources and life forms. If the nutritional basis is evaluated over the life time of the orchid, several different fungi could also be involved sequentially, utilizing different substrates. One such example is *Gastrodia elata* Bl. whose seedlings develop with the saprotroph *Mycena osmundicola* Lange and whose subsequent development depends on the parasite *Armillaria mellea* (Vahl.Fr.) Karst. (Xu and Mu, 1990).

The general picture is thus complex, the orchids utilizing a great diversity of fungi with different nutritional strategies, and new research, particularly based on DNA-identification, seems to be widening the range of orchid mycobionts. Saprotrophs are possibly overrepresented in the earlier records because they usually are easy to keep in pure culture. It is still premature to say whether orchids with certain mycotrophic strategies depend on fungi with a particular trophic strategy. Ectomycorrhizal or mildly pathogenetic fungi may represent a rather stable nutrient source, provided that the host is a long-lived tree, which could explain why several of the most chlorophyll-deficient orchid species have established that type of association. This is not without exceptions, however (Table 1). Saprotrophic fungi growing on plant debris are a more transitional energy source, especially in the tropics, and hence more suitable for opportunistic orchid species with a short life span and high recruitment rate and for species with a low dependency on fungi in their adult stage. Orchids relying on saprotrophs could improve their chances of longevity by a low degree of specificity towards mycobionts.

Mycorrhiza as a factor in orchid recruitment and distribution

Orchid mycorrhiza has an impact on plant fitness from germination through seedling stage and in many cases throughout life. The great seed production in orchids suggests that the mortality of seeds and seedlings is exceedingly large. Unsuitable substrate and ad-

verse physical conditions are challenges to any viable seed, but orchid seeds have the additional problem of locating a compatible mycobiont. Depending on the requirements of the fungus in question, the proximity of certain plants or debris of a particular kind may, by providing substrate for an appropriate fungus, be decisive for a successful establishment of seedlings. This challenge may persist in older plants that need to be reinfected every year.

The existence of fungal exudates that stimulate the rate of germination as well as the percentage, is well established *in vitro* (Rasmussen et al., 1998; review in Rasmussen, 1995). Extremely lean wood or soil based media are recommended if an indication of the natural situation for germination is to be obtained (for instance water agar amended with 0.5% ground wood, Whigham et al., unpubl.). In some cases, there is an all-or-none reaction *in vitro,* as in *Encyclia tampensis* Small, *Liparis liliifolia* A.Rich ex Lindl., or *Taeniophyllum obtusum* Bl. which only germinated in the presence of a fungus (Irawati, 1993; Rasmussen and Whigham, 1998b; Zettler et al., 1999). Recently developed techniques for field sowing *in situ* and retrieval of seeds (Masuhara and Kutsuya, 1994; Rasmussen and Whigham, 1993; van der Kinderen, 1995, Zelmer et al., 1996) have opened new possibilities for analysing germination behavior, fungal availability and natural substrates as they affect orchid recruitment. Some species, such as *Goodyera pubescens* R.Br., germinate freely in the ground without infection but the seedlings are not always successful in establishing mycorrhiza and high seedling mortality follows. Others, such as *Corallorhiza odontorhiza* Nutt. and *Liparis lilifolia*, are invariably infected when they are found in germinated condition (Rasmussen and Whigham, 1998b). Hence, the two latter species depend more on fungi during the germination process than *G. pubescens*, consistent with observations *in vitro*.

The reaction of the seed to fungi, however, is fairly unspecific and germination may be stimulated by less than optimum mycobionts and even in some cases by fungi and other microorganisms unable to participate in OM. Symbionts that are not fully compatible may result in high seedling mortality (Zettler et al., 1999). High germination percentage *in vitro* reflects physiological compatibility which does not necessarily apply in natural situations. For instance, Masuhara et al. (1993) germinated *Spiranthes sinensis* Ames with strains of *Rhizoctonia solani*, although the regular mycobiont appears to be *Tulasnella deliquescens* (Juel)Juel (Table 1) and Umata (1997 a ,b) was able to germinate *Erythorchis ochobiensis* with various fungi, with which the plant is not known to associate in nature. Further development of the seedling, however, often requires a narrower range of fungi than germination.

How fungi affect the seeds before actual invasion of the tissues remains conjectural. Ethylene and auxins are produced by some orchid mycobionts and are stimulatory to some seeds when added *in vitro* (Miyoshi and Mii, 1995; Rasmussen, 1995; Wilkinson et al., 1994). External addition of kinetin also often breaks dormancy of seeds *in vitro* (Miyoshi and Mii, 1998) but cytokinin production has not been detected in orchid fungi.

Burial of seed packets in prepared soil/wood mixtures showed that decaying wood is important to the germination and establishment of mycorrhiza in some orchids, and the species of wood and stage of decay influence the outcome (Rasmussen et al., 1998; Whigham et al., MS). The wood substrate increased the speed of germination even in asymbiotic controls, hence the seeds of these species seem to have developed the ability to react positively to a substrate that is likely to provide an appropriate mycobiont. Unsuitable substrate may be the reason why the immediate vicinity of mature plants – and their mycobionts – does not always appear to be suitable for seed germination. Masuhara and Katsuya (1994) thus found no connection between proximity of adult *Spiranthes sinensis* and success of seed packets in producing seedlings, and McKendrick et al. (2000a) made a similar observation in *Corallorhiza trifida*. Furthermore, seedlings of *Tipularia discolor* Nutt. were consistently found on decaying logs and stumps (which would eventually disappear) while plants in flowering stage were always found on the forest floor (Rasmussen and Whigham, 1998a). These examples suggest a dependency on successional vegetation for optimum seed-fungus interaction and seedling development.

Obligate associations of a species of orchid to a particular species of tree have been reported. The epiphytic species *Lepanthes caritensis* Tremblay and Ackerman was found only on *Micropholis guyanensis* in combination with thick moss cover (Tremblay et al., 1998), and the climber *Erythorchis altissima* Bl. almost exclusively occurred on the tree *Castanopsis sieboldii*, especially on dead trunks, often together with the shiitake mushroom, *Lentinus edodes* Berk. (Umata et al., 1994). The reason could either be that the tree in question is a living host, or an ectomycor-

rhizal partner, for the mycobiont, or that dead wood or litter of the tree offer a specialized substrate. Indirect effects of the tree on microclimate and soil composition are also possible.

Perkins and McGee (1995) found that colonies of *Rhizoctonia solani* associated with *Pterostylis acuminata* R. Br. extended up to about 0.5 m from the plants. This, as well as heterogeneity of the substrate, contribute to the patchiness of germination found in all succesfull recent field sowing experiments. Distribution of seed packets along transect lines showed that germination (and hence mycobiont presence) was positively correlated with sites of adult plants, with high content of non-decomposed organic matter, and with a low content of free nutrients (Batty et al., 2001).

Life forms, life history and phenology of orchid mycorrhiza

Previous reports that epiphytic orchids have low intensity of infection, compared to terrestrial species (Hadley and Williamson, 1972), tend to be contradicted by more recent data (Goh et al., 1992; Rivas et al., 1998; Senthilkumar et al., 1998, 2000 and references therein). These reports cover a considerable seasonal and local variation as well as variation among species. Aerial roots in epiphytic species generally are devoid of infection and roots in contact with the substrate extensively infected (Goh et al., 1992). Among temperate species, tuberous orchids appear to be more mycorrhized than rhizomatous species (Tatarenko, 1995).

Orchid mycorrhiza is present throughout the year in the tropical epiphyte *Vanda tessellata* Hook. ex G.Don with most mycophagy taking place while the plant is in active growth (Shagufta et al., 1993). Seasonal patterns of mycorrhization in temperate species mainly follows the phenology of the mycorrhized organs (Masuhara and Kutsuya, 1992; Rasmussen and Whigham, unpubl.) and is not linked to the seasonality of the photosynthetic structures.

The dependency of the plant on fungi indisputably changes over the lifetime of the orchid; in most orchids a decrease is evident from the seedling stage onwards. Some species remain heavily mycorrhized, in spite of photosynthetic capacity of the leaves (Rasmussen and Whigham, unpubl.). Zelmer et al. (1996) found that seedlings associated with a wider range of fungi than older plants did. This might indicate a change of mycobionts during development of the individual plant; however, it might also reflect a fairly unspecific germination with subsequent decimation of seedlings, leaving only those with optimum symbiosis to develop into adults. In the case of *Gastrodia elata*, however, a shift in mycobiont seems to be the rule, from *Mycena osmundicola* to *Armillaria mellea* (see above, Xu and Mu, 1990). It is known from symbiotic propagation programs that the pelotons extracted from roots of adult plants often have no positive effect on seedling development *in vitro* which suggests that it is common for adult orchids to contain more fortuitous infections. Dijk et al. (1997) suggest an adaptive advantage of a symbiont shift during the life history of an orchid. A seedling which has established with a saprotrophic mycobiont on a carbohydrate-rich substrate may find the substrate gradually changing over time. This appears to be the case in *Tipularia discolor* (see above, Rasmussen and Whigham, 1998a). While a slow growing, weak competitor with a specialized substrate requirement may be an optimum mycobiont in early life of the orchid (or in a short-lived species), a more aggressive fungus with an extensive mycelium and generalized substrate utilization might be a better support for the adult orchid in mature vegetation.

Identification of mycobionts

Pure cultures based on isolations from orchid roots, preferably from single pelotons, have up till now been the main source of information about orchid symbionts. The fungal partner in OM is always a basidiomycete and the isolates generally are sterile mycelia with very little tendency to sporulate in culture. Certain mycelial characters, particularly the ultrastructure of the septal pore (Currah and Sherburne, 1992), the size and shape of monilioid cells, formation of sclerotia, and enzyme activity are useful as distinguishing characters in sterile mycelia of *Rhizoctonia sensu lato* (Zelmer and Currah, 1995b), which comprise most of the orchid mycobionts now known. However, the mycelia generally do not yield many distinguishing characters that enable identification below generic level.

A much finer distinction between strains is now possible by means of DNA analysis of infected orchid tissue. These techniques are mostly based on PCR with fungal primers on macerated orchid tissue. This reaction will amplify selected parts of the fungal genome for further characterization of the DNA either by sequencing or fragmentation patterns. The level of dis-

tinction between strains depends on the variability of the part of the genome under study. Provided corresponding DNA from suitable identified reference fungi have been processed, a phylogenetic analysis will place the unknown strain in a taxonomic group. Hopefully, these techniques will eventually reveal the full diversity of orchid symbionts, including those whose nutritional requirements cannot easily be met in pure culture. DNA analyses of infected tissue, however, holds the same kind of drawbacks as pure cultures obtained from plating tissue fragments, i.e. that mixed infection and fortuitous contaminants can obscure results. Since peloton formation is an important criterion for an orchid symbiont, DNA-analysis based on single pelotons extracted from fresh or dried orchid tissue is a new promising approach (Kristiansen et al., 2001).

Many OM isolates are referred to the form genus *Rhizoctonia* in sterile condition. Roberts (1999) lists 15 species with *Rhizoctonia* anamorphs that are known to be orchid symbionts: 5 species of *Ceratobasidium*, 3 species of *Thanatephorus*, 1 species of *Serendipta* (*Sebacina*) and *Oliveonia*, and 5 species of *Tulasnella* (Table 1). These all belong in Heterobasidiomycetes.

Agaricanae are represented among orchid symbionts by *Armillaria* (Cha and Igarishi, 1996; Lan et al., 1994; Terashita, 1996) and *Mycena* (Fan et al., 1996; Lan et al., 1996), 'Hericianae' and 'Thelophoranae' are represented by Russulaceae and Thelephoraceae (McKendrick et al., 2000a; Taylor and Bruns, 1997), and 'Hymenochaetanae' by *Erythromyces* (Umata, 1995, 1998a). Five major taxonomic groups are thus represented among OM fungi (Table 1).

The relevance of Mycelia Radices Atrovirens (MRA) that are sometimes isolated from orchid tissue (Currah et al., 1990) is uncertain. MRA fungi have not been successfully used for germination *in vitro* and they do not appear to form pelotons. Possibly they are fortuitously present within the roots or superficial contaminants that arise in cultures produced from incubating slices of surface sterilized roots. This isolation procedure is widely used (e.g., Currah et al., 1990; Vertenyi and Bratek, 1996), but not as reliable as single-peloton isolations.

Specificity between orchid and mycobiont

It is necessary to distinguish between associations that may be viable under certain experimental conditions, as *in vitro,* and thus showing a *physiological compatibility* and those association that are possible and competitive under natural conditions, i.e. a *specificity* in ecological context. Ideally, specificity should be tested either *in situ* or under realistic climate conditions *in vitro*, with the mycobiont growing on a near-natural substrate, and with fully viable, non-dormant seeds; furthermore, a full identification of the species participating is essential. Most often, however, our evidence is based on case studies quite far from that ideal.

Even so it has become clear that orchids and fungi do associate according to some compatibility barriers, though usually not on a species-to-species level. The nature of these barriers is still entirely unknown. A narrow specificity in the orchid could be a reason for rarity and vulnerability of the plant species, just as a narrow food preference would in an animal species, and so is an issue of conservational interest. One suggestive example is *Corallorhiza mertensiana* Bong., a rather rare species (Freudenstein, 1997) which was found to associate with a narrower range of mycobionts than the wide-spread relative *C. maculata* (Taylor and Bruns, 1999). However, very little is known at present about how the symbiosis with fungi affects competition, survival and distribution of orchids and we have not even begun to consider how the relationship may affect the fungal community.

Germination may be stimulated by a range of fungi, with little bearing on their compatibility or actual relationship with the plant in nature. Adult plants may also house a variety of fungi. Hence, the holomycotrophic seedling phase appears as the bottleneck stage at which tolerance towards suboptimum symbioses is lowest. It is also the life history stage at which mortality is probably the highest (Rasmussen and Whigham, 1998b). A balanced seedling development may thus be the best criterion for compatibility.

Even with unidentified mycobionts it is often possible to ascertain that orchid species under standardized (if fairly artificial) conditions differ in symbiont preference: Tomita and Konno (1998) showed that *Aorchis cyclochila* (Franch. and Sav.) T.Hashimoto, *Dactylorhiza aristata* (Fisch. ex Lindl.) So, *Gymnadenia camtschatica* Miyabe & Kudo had other preferences for fungi than *Amitostigma kinoshitae* (Ohwi & Hashimoto) Hashimoto and *Ponerorchis graminifolia* Rchb.f.; *Cypripedium macranthos* Sw. appeared to have a narrower endophyte range than the rest. Comparative studies of sympatric or closely related species are more illustrative. For instance, Zettler and Hofer (1998) noted that a strain of *Epulorhiza*

inquilina Currah et al., obtained from *Platanthera clavellata* (Michx)Luer, was more efficient *in vitro* in promoting seedling development of that species than isolates from three other co-habiting species of *Platanthera*. This suggests that sympatric orchid species avoid mutual competition for food by employing different species of fungi. This pattern is also evident in *Pterostylis acuminata* compared to other surrounding orchid species (Perkins and McGee, 1995) and in comparison of two species of *Corallorhiza*. In the latter case, although all the symbionts of both orchid species were placed in Russulaceae, samplings over a wide geographic range did not show any sharing of fungal species (Taylor and Bruns, 1999).

Constant yield of the same fungus or group of fungi from repeated sampling of an orchid species over a geographic range supports the assumption of a large degree of specificity. For instance, 14 samples from *Cephalanthera austinae* over a wide geographic range all belonged to Thelephoraceae, and 18 samples from *Corallorhiza maculata* were all referred to Russulaceae (Taylor and Bruns, 1997). Isolations from *Galeola septentrionalis* Rchb.f. consistently yield a range of species belonging to *Armillaria* (Terashita, 1996). Collections of *Erythrorchis ochobiensis* always contained *Erythromyces crocicreas*; in spite of that, this orchid could germinate *in vitro* and develop mycotrophic seedlings with a diversity of fungi: *Ganoderma australe* (Fr.) Pat., *Loweporus tephroporus* (Mont.) Ryv., *Microporus affinis* (Fr.) Kunt., *Phellinus* sp., *Auricularia polytricha* (Mont.)Sacc. and *Lentinula edodes* (Umata, 1997a, 1998a,b). These physiological symbionts do not appear to be ecological symbionts. Even physiological compatibility has its limits in *Erythrorchis*, though: seeds tested with *Lyophyllum shimeji* (Kawamura) Hongo germinated but without peloton formation or normal seedling development, and seeds incubated with *Tricholoma fulvocastaneum* Hongo did not germinate at all (Umata 1997b).

Dijk and Eck (1995) found compatibility of *Anacamptis morio* (L.)Batem. et al. with *Epulorhiza* sp., but not with *Ceratorhiza* sp.; with three species of *Dactylorhiza, Ceratorhiza* showed greater efficiency than *Epulorhiza* but also less tolerance to high N concentrations. Within the genetically determined range of possible mycobionts, geography and habitat conditions may influence which one(s) is active in a particular population (Taylor and Bruns, 1999).

Interactions with other micro-organisms

Germination of either *Caladenia latifolia* R. Br. or *Diuris magnifica* D.L.Jones on mixed or single inoculations suggests that competition between the OM fungus and other fungi can be a significant factor in nature (Quay et al., 1995). Synergy between microorganisms in OM is also possible; bacteria associated with *Pterostylis vittata* Lindl. stimulated symbiotic germination, probably because they produced IAA or induced the plant into auxinproduction (Wilkinson et al., 1994). These bacteria belonged to *Pseudomonas putida* (but not all strains), *Zanthomonas maltophilia* and *Bacilllus cereus*, whereas other bacterial strains were ineffective.

Structure and ultrastructure

Traditionally, two types of orchid mycorrhiza have been recognized, i.e. tolypophagy, found in the great majority of species, and ptyophagy, only noted in a number of highly mycotrophic tropical orchids (Rasmussen, 1995, and references therein). In 1995 the most recent reference to ptyophagy dated from 1936, and ptyophagy remained an obscure phenomenon. However, recent studies throw new light on this type of orchid-fungal symbiosis.

In *Gastrodia elata*, the hyphae of *Armillaria mellea* extend in bundles along the roots in cortical canals (Wang et al., 1997). These canals develop from lines of 'passage cells' whose adjoining cell walls and original cell content deteriorate. The outer cortex, outside the passage canal consists of 'host cells' where the hyphae coil and apparently persist, and the inner cortex contains 'digestion cells' (Figure 1). When hyphae enter a digestion cell, an interface is formed between the receding plant plasmalemma and the hyphal wall. Electron transparent vesicles with lysosomic (or fungistatic?) properties pass the perihyphal plant plasmalemma and are released into the interface (Figure 2). Subsequently, electron-dense vesicles appear along the plant plasmalemma and elongate to form a radiating tubular system around the hyphae. These vesicles are believed to be endocytic ('endocytic tubes') and contain products from hyphal dissolution; they appear to cut off minor vesicles that migrate into the plant cytoplasm (Figures 3 and 4). Fusion between these and electron-transparent, presumably lysosomic vesicles scattered in the cytoplasm is believed to mark the final step in the breakdown of the liquid hyphal

products (Figure 5). The fungal wall shatters in the interfacial space (or digestion vacuole). Invaginations from the plasmalemma, to which electron transparent vesicles and parts of ER fuse, appear to pinocytose chips of fungal wall for further breakdown (Figures 6 and 7).

According to Burgeff (1936), the distinguishing element in ptyophagy is that fungal tips of the intracellular hyphae become lysed and the hyphal cell contents thus released, whereas in tolypophagy there is an overall collapse and breakdown of hyphae. In view of the electron microscopic evidence now available this discription may need to be modified. However, there is little doubt that ptyophagy differs rather markedly from the more well-known tolypophagic pattern, both at the histological level (the formation of passage canals) and in the ultrastructure of the digestion process (endocytic tubes, pinocytosis of hyphal wall fragments). The clumps of collapsed and aggregated hyphal walls, so characteristic of tolypophagy, seems to be lacking. Some differences could be due to properties of the mycobionts in question, others to the plant. However, ptyophagy adds to the diversity of plant/fungal interactions, apparently in a rather outstanding manner and deserves further study.

The tolypophagic interaction is comparatively well investigated at the ultrastructural level, but some unsolved questions remain: The origin of the interfacial matrix, the actual transfer of fungal products to the plant cytoplasm across that barrier, the mechanisms in cells, where hyphae are present without being digested, and in cells where hyphae are rejected, and finally the exportation of mycotrophic products from digestion cells into the stele (Rasmussen, 1995).

A cytochemical localization of adenylate cyclase activity showed accumulation along the plasmalemma at the plant cell walls in both infected and non-infected cells, but not along the membrane invaginations enveloping invading hyphae. While these membranes were clearly derived from the plasmalemma, their properties changed in contact with the hyphae (Uetake and Ishizaka, 1995). Adenylate cyclase catalyses the formation of cyclic AMP, presumed to be intracellular messenger molecule. Its functions and possible role in preventing or promoting hyphal invasion are uncertain.

The interfacial matrix was shown by Peterson et al. (1996) to contain pectins, cellulose and $\beta 1$–3 glucans when the peloton hyphae are collapsing but none of these substances were detected before peloton breakdown.The cortical microtubule system (MT) disappeared in cells during infection but short MT's were observed between hyphae in colonized cells, forming a network through the peloton and connecting to the nucleus. During lysis MT's were observed between hyphae within and around the collapsing peloton (Uetake et al., 1997; Uetake and Peterson, 1998). The fact that other researchers did not observe MT's during infection (Dearnaley and McGee, 1996) could be due to a different fixation and microscopy technique by which short MT's could easily be overlooked. Also actin filaments (AT) were rearranged during infection into a network radiating from the perihyphal membrane towards the cell walls. This network remained during peloton lysis, but the cortical AT system subsequently reappeared (Uetake and Peterson, 1997). Both MT's and AT's in digestion cells seem to stabilize the peloton and possibly guide vescicles and other organelles towards and away from the interface. The AT system found in infected roothair cells, however, was oriented along the longitudinal axis of the cell (Uetake and Peterson, 1997) and could thus act as a guide leading the hyphae towards the protocorm body.

The content of digestive enzymes is much higher in infected tissues than in uninfected, and by histochemical localization Senthilkumar et al. (2000) implicated peroxidase, glutamate dehydrogenase, esterase as well as malate dehydrogenase in the lysis of pelotons.

Physiology of orchid mycorrhiza

The control of hyphal invasion in orchid tissues has traditionally been attributed to the production of phytoalexins, known from tubers and rhizomes of orchids; only recently has the phytoalexin orchinol also been demonstrated in protocorms (Beyrle et al., 1995). Fungal invasion and wounding of orchid tissues induce the production of phytoalexins (Gehlert and Kindl, 1991; Reinecke and Kindl, 1994a, b).

The balance between the symbionts is affected amongst other things by the source of nitrogen (Beyrle et al., 1995), so that a low supply coinciding with a high availability of carbohydrates gave a balanced mycorrhiza in *Anacamptis morio*, whereas high supply of nitrogen and carbohydrates resulted in rejection of the fungus. Any combinations of with low carbohydrate supply resulted in parasitism of the fungus on the plant. Shortage of external carbohydrate sources tended to increase fungal virulence. High nitrogen availability could lead to rejection of the fungi which

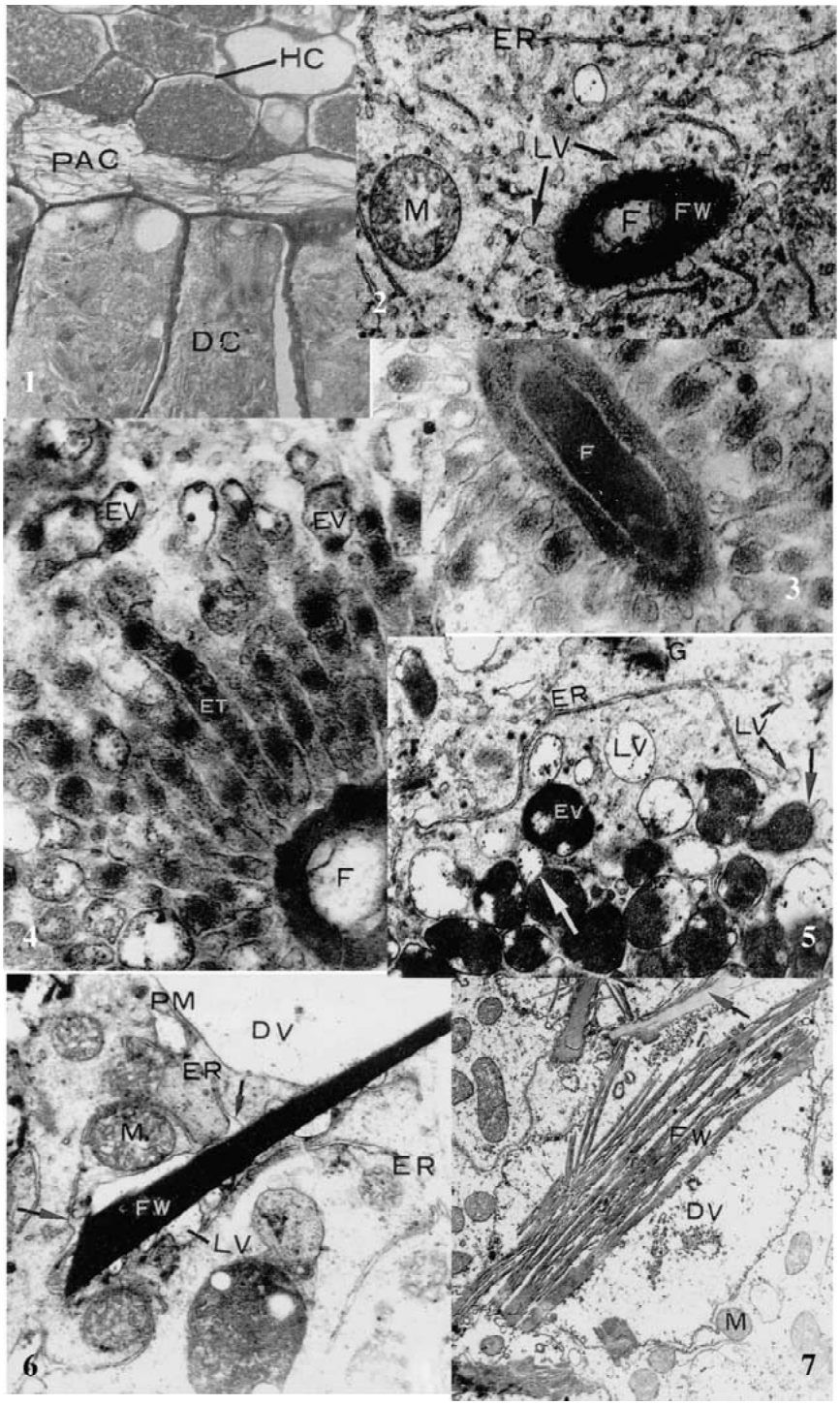

Figure 1. Longitudinal section of an infected root of *Gastrodia elata* showing passage canal (PAC), host cells (HC) and digestion cells (DC). (2) Digestion cell showing abundant ER, electron-transparent lysosomal vesicles (LV) fusing into the space around the thickened wall of invading hypha (F). (3) Endocytic vesicles forming aroung hypha (F) in early stage of fungal disintegration. (4) A radiating system of endocytic tubes (ET) forms around hypha, and cut off endocytic vesicles (EV), presumably filled with fungal products. (5) Endocytic vesicles (EV) fusing (white arrow) with ER-produced lysosomal vesicles (LV). (6) LV and ER fusing with perifungal plasmalemma to produce a large digestion vesicle around the remains of fungal wall material. Larger pieces of wall material (FW) partly enveloped by digestion vacuole membrane in the process of breakdown to smaller parts. (7) Digestion vacuole with finely chipped hyphal wall remains. Arrow points to larger piece, as in Figure 6. Magnifications: 1: × 120, 2: × 20000, 3: × 24000, 4: × 26000, 5: × 26000, 6: × 30000, 7: × ca. 15000. From Wang et al. (1997) printed with permission from Acta Botanica Sinica.

was accomplished by thickening of plant cell wall and accumulation of phenolics (Beyrle et al., 1995). Dijk and Eck (1995) noticed a negative effect of high nitrogen supply on protocorm yield *in vitro*, when the mycobiont was a representative of *Ceratorhiza* but could not detect any adverse effects when *Epulorhiza* sp. was involved. This is consistent with field observations that populations of *Dactylorhiza majalis* (Rchb.) Hunt & Summerh. responded negatively to fertilization with nitrogen, as well as phosphorus and potassium (Dijk and Olff, 1994), and not only as a result of intensified competition from the surrounding vegetation. Thus orchid mycorrhiza seems more competitive on poor soils which is consistent with many field observations. Symbiotic seedlings *in vitro* reach a higher nitrogen concentration in their tissues than asymbiotic controls which confirms that the mycobionts assist in nutrient uptake for the plants (Lee et al., 1997).

The root/shoot ratio increased, and leaf development was inhibited, in a *Cattleya* hybrid grown *in vitro*, when external carbohydrate was added (Beyrle and Smith, 1993a). In *Anacampsis morio*, greening of leaves was prevented by high carbohydrate concentration in the substrate (Beyrle and Smith, 1993b). Carbohydrate availability could thus be one of the factors determining the trade-off between photo- and mycotrophism. Another factor could be light, since exposure to light is required by some species before leaves develop from primordia (Zettler et al., 1995). The transfer of soluble carbohydrates from the mycorrhizal fungus to the heterotrophic plant has been further corroborated by a recent study: labelled glucose was traced from the mycelium of *Mycena osmundicola* into the seedlings of *Gastrodia elata* and labeling subsequently appeared in meristematic (non-infected) tissues of the plant (Lan et al., 1996). However, traces of label were observed in rhizomorphs of *Armillaria mellea* after feeding *Gastrodia elata* with labeled glucose (Lan et al., 1994). This is the first ptyophagic relationship to be studied with respect to nutrient exchange and it is possible that it differs from tolypophagy, so that a limited flow of carbohydrates towards the fungus is possible. However, the observation might also indicate a senescence phenomenon. There is little doubt that the general carbohydrate flow occurs in the direction of the clearly chlorophyll-deficient *G. elata*.

Mycorrhizal infection seems to enhance the uptake of water since seedlings of both the terrestrial species *Platanthera integrilabia* (Correll) Luer and the epiphyte *Epidendrum conopseum* R.Br. had higher water content than non-infected controls (Yoder et al., 2000). This would seem a particularly valuable feature in the epiphytic and epilithic life forms of orchids.

Ex situ applications of the mycorrhizal association

The study of orchid mycorrhiza was founded almost 100 years ago by Bernard (1904, 1909) and Burgeff (1909, 1932, 1936), but since interest in the subject was revived about 20 years ago, new insight has been accumulating and, perhaps more important, application to horticulture and conservation has begun. Several conservation projects utilize symbiotic techniques to propagate plants, to bank seeds and fungal cultures, and to evaluate natural growing sites (e.g. Dixon, 1994; Stewart, 1993). Rare and endangered species of orchids have been propagated symbiotically with the purpose of *ex situ* conservation or reintroduction (e.g. Zettler and McInnis, 1992).

Orchids are mostly grown commercially for their ornamental value but other uses exist; the symbiotic cultivation of *Gastrodia elata* has become an important enterprise based on the medicinal use of the tubers (Xu and Mu, 1990), and the edible rhizomes of *G. cunninghamii* Hook.f. are reported to be a delicious vegetable (Harris, 1997). Symbiotic cultivation techniques widen the range of species that can be grown profitably.

Maintaining a living orchid collection may present considerable difficulties; many species of orchids still defy cultivation except in seminatural conditions in botanical gardens. Most holomycotrophic taxa are deemed to be impossible to grow. The likely reason is that orchid species with a large dependency on their mycobiont have difficulties in establishing or maintaining a functional symbiosis in a garden or greenhouse environment. The survey by Goh et al. (1992) comparing collections from nature and cultivated plants growing in garden beds or pots clearly showed that mycorrhization was low in pot culture in commercial nursery potting mixtures. Rivas et al. (1998) found that although most cultivated plants under seminatural conditions were extensively colonized by mycorrhizal fungi, a few introduced species (2 out of 24) apparently were sparsely mycorrhized. Pest control by means of some fungicides of course presents a special problem to symbiotic orchid cultures (Kummuang, 1997).

Large scale symbiotic propagation may take place on complex carbohydrate substrates such as sawdust

(Umata, 1997a, 1998a), either inoculated by known orchid endophytes or amended with soil samples from natural orchid sites (Johnson, 1994). Inoculates of orchid mycobionts for larger-scale inoculation may be produced by letting the fungi invade sterilized expanded clay particle that can subsequently be dried and ground before application to soil or plant growing media (Beyrle et al., 1989). Techniques are now developing for producing seeds for commercial production or conservation encapsulated with suitable inoculum to secure an initial compatible symbiosis (Tan et al., 1998; Wood et al., 2000).

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