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REVIEW

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Interactions between *Pisolithus tinctorius* and its hosts: a review of current knowledge

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Abstract *Pisolithus tinctorius* (Pers.) Coker and Couch [Syn. = *P. arhizus* (Scop.: Pers.) Rauschert] (*Pt*) is a widespread ectomycorrhizal basidiomycete forming mycorrhizas with a variety of hosts. Developmental and functional aspects of the symbiosis are well documented and thus *Pt* has been adopted as a model organism for investigations of the molecular basis of ectomycorrhizal interactions. In this review of the current state of knowledge of interactions between *Pt* and its hosts we demonstrate that *Pt* displays much intraspecific heterogeneity of host specificity, physiology and the benefits the fungus can impart upon the host plant. It is not clear at present how far such heterogeneity reflects systematic segregation within *Pt*.

Key words *Pisolithus tinctorius* · *Pisolithus arhizus* · Fungus – root interactions

Introduction

Pisolithus tinctorius (Pers.) Coker and Couch [Syn. = *P. arhizus* (Scop.: Pers.) Rauschert] is an ectomycorrhizal (ECM) gasteromycete with a widespread global distribution (Marx 1977). Having been championed during the 1970s for use in forestry inoculation programmes, for which a number of inoculation protocols were developed (reviewed by Marx and Kenney 1982), a considerable body of literature exists relating to host plant responses to *Pisolithus* infection under a range of conditions. The ease with which the fungus can be grown *in vitro* has facilitated extensive study of its physiology and the simplicity of mycorrhiza synthesis under controlled conditions with a range of host plants has ensured full documentation of the ontogeny and ultrastructure of *Pisolithus* ECM. Detailed information available on the development, compatibility and physiology of the symbiosis has made the *Pisolithus* – *Eucalyptus* interaction the preferred system for investigation of the molecular basis of the fungus – root interaction (Tagu and Martin 1996). In this review, we attempt to bring together information on the interaction between a single ECM fungus (*Pisolithus*) and its hosts. It was not our intention to cite every published paper relating to the fungus, but rather to concisely review current knowledge and to highlight the gaps in our knowledge. Only by understanding and integrating aspects of the taxonomy, development, physiology, ecology and molecular biology of the interactions between a single fungus and its hosts can a full understanding of this model ECM interaction be attained.

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The fungus

Although considerable heterogeneity exists in terms of carpophore, spore and isolated culture morphology, taxa within the genus *Pisolithus* are widely regarded as conspecific (Coker and Couch 1928; see Watling et al. 1995). Several separate *Pisolithus* species, including *P. kisslingi* E. Fisch, *P. pusillum* Pat. and *P. aurantioscabrosus* Watling et al. have, however, been described in tropical South East Asia, based on distinctive carpophore and basidiospore morphology (Watling et al. 1995). Because individuals within *P. tinctorius* (*Pt*) display considerable variation in carpophore and basidiospore morphology, several species have been proposed within the group currently described as *Pt* (Bronchart et al. 1975; Calonge and Demoulin 1975). For example, within Australia, *P. microcarpus* (Cke. and Mass.) Cunn is regarded as a separate species (Cunningham 1942).

Recently analysed electrophoretic patterns of expressed mycelial proteins from *Pt* isolates collected from different geographical regions of Australia (Burgess et al. 1995b) indicate much variability in polypeptide pattern within *Pt* and a correlation between group-

ings based on polypeptide pattern and geographical origin. Several distinct groups of polypeptide patterns appear to exist even within the state of Western Australia. While the polypeptide grouping described by Burgess et al. (1995b) corresponded to differences in basidiospore type, further study of a wider range of isolates is required before a fuller picture of *Pisolithus* speciation in Australia can be developed. Recent unpublished data from our laboratory using a variety of molecular approaches (RAPD, ITS-RFLP, microsatellite and sequence analysis) indicates considerable polymorphism within *Pt* isolates collected from around the Sydney region, NSW, Australia (IC Anderson, SM Chambers, JWG Cairney, unpublished data).

Pt has been recorded in a range of habitats including forest, urban and orchard sites, as well as eroded and mine-site soils (Marx 1977; Malloch and Kuja 1979), but carpophores are more usually found in relatively dry sites with little humus or along roadside areas (eg. Castellano and Trappe 1991). Information from Western Australian reforestation sites indicates that the fungus is an early coloniser (Gardner and Malajczuk 1988) and it is generally regarded as poorly competitive with other ECM fungi (Marx et al. 1984; McAfee and Fortin 1986). It is perhaps for these reasons that *Pt* persists best in forestry inoculation programmes on sites subject to edaphic stresses (see below). Most, but not all, *Pt* isolates (see for example Reid and Woods 1969) produce an extensive extramatrical mycelial phase which in many cases differentiates into linear organs (Kammerbauer et al. 1989; Agerer, 1991; Lamhamedi and Fortin 1991). Based on descriptions by these authors and adopting the nomenclature of Cairney et al. (1991), linear mycelial organs of *Pt* can be described as 'apically diffuse, simple rhizomorphs'. From what is known about the structure and function of rhizomorphs in other fungal systems, their formation may be important in channelling nutrients and water to and from the host and in protecting extramatrical mycelium against adverse environmental conditions (Thompson 1984; Cairney 1992). A recent population study indicates that mycelial individuals (= genets) of *Pt* in the field can exceed 30 m in diameter (Anderson et al. 1997).

Pt also produces sclerotia as part of its extramatrical mycelial phase (Dennis 1980; Piché and Fortin 1982; Fortin et al. 1983). While environmental conditions may influence the shape and structural detail of sclerotia (Grenville et al. 1985), in all cases they comprise an external melanised rind surrounding a cortex and medulla (Dennis 1980; Piché and Fortin 1982; Grenville et al. 1985). In common with other fungal sclerotia, histochemical staining indicates that the cortex and medulla are rich in protein, lipids and carbohydrate, implying a storage function and the ability to allow *Pt* to withstand edaphic stresses in a vegetative state (Piché and Fortin 1982; Grenville et al. 1985). The production of sclerotia, along with aggregation of extramatrical mycelium into rhizomorphs, may thus be important in the reported success of *Pt* in stressful soil conditions.

Ectomycorrhiza formation

Host–fungus specificity

Laboratory synthesis experiments have confirmed that *Pisolithus* forms ECM with a range of host plant genera (see Table 1). There is, however, considerable evidence of specificity in the interaction between host root and *Pt.* It has been frequently noted, for example, that *Pt* isolated from carpophores collected in association with *Pinus* spp. are poor colonisers of *Eucalyptus* spp. (eg. Chilvers 1973; Malajczuk et al. 1990; Lei et al. 1990a; Burgess et al. 1994). Even isolates from different conifer species are not necessarily intercompatible (Marx and Bryan 1970; Marx 1981). Further, some *Pt* isolates show ECM compatibility with clones derived from mature eucalypt trees, but are poorly compatible with clonal host plants generated from young seedlings, suggesting that the developmental maturity of host material can also influence compatibility (Tonkin et al. 1989). A recent, detailed study of interactions between 20 *Pt* isolates from different geographical regions and *Eucalyptus grandis* (Burgess et al. 1994) indicates wide variation in both rate and extent of ECM formation by different isolates. The extent of ECM formation varied from a fully developed sheath and Hartig net in compatible isolates through isolates that formed only a superficial sheath, to isolates that formed no identifiable ECM structures. Incompatibility between *Pt* and host roots can also be expressed in polyphenol accumulation in host tissue and thickening of host cell walls abutting the incompatible isolate (Tonkin et al. 1989; Lei et al. 1990b). The data of Burgess et al. (1994) are also signif-

Table 1 Host genera with which *Pisolithus* spp. isolates have been confirmed as forming ectomycorrhizas in mycorrhizal synthesis experiments

Genus	Source
Abies	Marx 1977
Acacia	Ba et al. 1994
Aflezia[a]	Ba and Thoen 1990
Allocasuarina	Theodorou and Redell 1991
Alnus	Godbout and Fortin 1983
Arbutus[b]	Zak 1976
Arctostaphylos[b]	Molina and Trappe 1982a
Betula	Marx 1977
Carya	Marx 1977
Castanea	Martins et al. 1996
Castanopsis	Tam and Griffiths 1994
Casuarina	Theodorou and Reddell 1991
Eucalyptus	Marx 1977
Hopea	Yazid et al. 1994
Larix	Molina and Trappe 1982b
Pinus	Marx 1977
Populus	Godbout and Fortin 1985
Pseudotsuga	Marx 1977
Quercus	Marx 1977
Tsuga	Marx 1977

[a] No Hartig net formed, only a sheath
[b] Arbutoid mycorrhizas

icant in showing intraspecific variation of intercompatibility with *Pt* isolates within the genus *Eucalyptus*. Furthermore, the isolates used in their study were derived, not only from a variety of geographical origins, but also from carpophores with different morphological characteristics. The extent to which such characteristics reflect taxonomic variation within *Pt* remains unclear (see above), but it further highlights the need for detailed systematic studies.

Genetics of the interaction

In vitro germination of spores collected from mature *Pt* carpophores can be achieved within a few weeks under axenic conditions, and has permitted investigation of aspects of the importance of *Pt* genetics in the fungus–plant interaction. Successful germination requires either the presence of a germination activator (in the form of a yeast colony) (Bulmer 1964; Lamb and Richards 1974) or a host plant seedling (Kope and Fortin 1990). Germination rates are at best 0.38% (Lamb and Richards 1974), but can be much lower in the absence of activator colonies (Kope and Fortin 1990). Based on spores isolated from carpophores originating in North America, South Africa, Australia and Europe, it appears that tetrapoplar incompatibility (four mating types) exists within *Pt* monokaryons (Kope and Fortin 1990; Rosado et al. 1994b). Crosses between some monokaryons and dikaryons have also been achieved in the laboratory (Kope 1992). Both monokaryons derived as single-spore isolates and reconstituted dikaryons are capable of ECM formation with *Pinus* spp. (Lamhamedi et al. 1990). Individual mono- and dikaryons derived from a single carpophore show differential affinities for mycorrhiza formation on *Pinus* spp., but *Pt* monokaryons are generally less efficient in ECM formation than dikaryotic mycelia (Lamhamedi et al. 1990). Dikaryosis is thus thought to be required for the full expression of ECM-forming abilities.

Reconstituted *Pt* dikaryons show variability in the growth form of the extramatrical mycelia, particularly in extension rates and the degree to which rhizomorphs are formed (Lamhamedi and Fortin 1991). They also show variable abilities to improve host plant growth, drought tolerance and mineral nutrient content (Lamhamedi et al. 1992a), although the extent to which these reflect the relative abilities of dikaryons to infect roots or differences in extramatrical mycelial characteristics remains unclear. In some cases, monokaryon crosses show additive abilities for ECM formation, in others the interactions are non-additive (Lamhamedi et al. 1990; Rosado et al. 1994b). Such variability may facilitate directed fungal breeding for enhanced ECM-forming abilities, particularly if coupled with the known potential for enhancing host tree receptiveness to *Pt* via a plant breeding programme (Rosado et al. 1994a).

Developmental aspects of the fungus-host interaction

The interaction between *Pt* and a host root begins prior to fungus–root contact. Diffusible substances released from the host appear to stimulate a chemotropic growth response of compatible *Pt* hyphae towards the host (Horan and Chilvers 1990). Within 1 day of the introduction of the fungus to a eucalypt root system, and prior to fungus–root contact, there is evidence of a chemical interaction between fungus and host in the form of a browning reaction in outer root cap cells (Horan et al. 1988). Shortly after contact, fibrils (believed to be glycoproteins of fungal origin) can be observed at the fungus–root interface in compatible but not incompatible *Pt* – host interactions (Piché et al. 1983; Lei et al. 1990b). The glycoproteins may be important in either recognition and/or attachment processes. *Pt* hyphae may invade moribund root cap cells at this stage, perhaps providing an essential nutrient source for the process of ECM morphogenesis (Horan et al. 1988; Stephanie et al. 1996).

Molecular investigations of the *Pt* – *Eucalyptus* interaction indicate an altered polypeptide expression in both partners, including the appearance of mycorrhiza-specific polypeptides ('ectomycorrhizins') during ECM formation (Hilbert and Martin 1988; Hilbert et al. 1991; Burgess et al. 1995a). Altered patterns of polypeptide expression are more apparent with *Pt* isolates that form ECM rapidly than in less-compatible isolates; indeed *Pt* isolates that do not form ECM with *Eucalyptus* spp. fail to alter expression patterns of host or self polypeptides (Burgess et al. 1995a). In particular, several acidic polypeptides appear to be enhanced in *Pt* during ECM formation. From the high level of synthesis that they display, these are regarded largely as structural (cell wall) fungal proteins. At the same time, a major cell wall mannoprotein is down-regulated, being almost undetectable in hyphae following root contact (Tagu and Martin 1996). During ECM formation there is a general down-regulation of host polypeptides, which is thought to reflect reduced root-system metabolism during symbiosis (Burgess et al. 1995a; Burgess and Dell 1996). While demonstrated changes in wall proteins are assumed to indicate a major role of the *Pt* cell wall in the fungus – root interaction (Tagu and Martin 1996), identification of the roles of up- and down-regulated proteins in the symbiotic interaction remains a major challenge for mycorrhiza research.

Changes in polypeptide patterns can be clearly identified within hours of symbiont contact, indicating that the molecular interaction begins prior to visible signs of ECM development (Hilbert et al. 1991). There is even evidence that some *Pt* polypeptides are up-regulated prior to fungus–root contact and that these may represent components of fungal surface glycoproteins observed during early *Pt* – root interactions (Burgess et al. 1995a). More direct evidence for altered gene expression during *Pt* – *Eucalyptus* ECM formation also exists. By screening cDNA libraries derived from free-living

and symbiotic material, Tagu et al. (1993) and Tagu and Martin (1995) showed enhanced transcription in *Pt* during ECM development. Several *Pt* cDNA transcripts encoding polypeptides in the hydrophobin family have been isolated (Tagu et al. 1996). This is significant since hydrophobins are thought to be involved in differentiation and/or adhesion processes in other fungal systems (Wessels 1994) and may be a critical component in establishment of the *Pt* – *Eucalyptus* ECM.

Although host polypeptides appear in general to be down-regulated during *Pt* ECM establishment, there are a number of reports that host root chitinase and peroxidase activities are stimulated during the early stages of *Pt* infection and that expression remains at a high level during development of the ECM organ (Albrecht et al. 1994a,b,c). Activities of both enzymes were shown to be higher during infection by highly compatible *Pt* isolates than in uninfected roots challenged by poorly compatible *Pt* isolates. Although Albrecht et al. (1994a) reported fungal extracts to elicit the chitinase response, Albrecht et al. (1994b) found that hypha–root contact was required for increased activity. While it is tempting to speculate on a role for chitinase and peroxidase activities in the infection process, possibly in differentiation of the Hartig net (Albrecht et al. 1994b), it must be noted that Hodge et al. (1995) observed no increase in chitinase activity in roots of either *Pinus* or *Eucalyptus* spp. during infection with a single isolate of *Pt*. The latter authors, however, provided no information about the efficacy of the isolate for ECM formation with either host. *Pt* infection has also been shown to induce a systemic chitinase increase in *Eucalyptus* spp., although this response was apparently independent of isolate aggressiveness (Albrecht et al. 1994c).

Pt typically forms bright yellow ECM with a thick fungal sheath and well-developed Hartig net, yet there are inter-isolate differences in the degree of sheath development (Marx et al. 1970). The bright yellow colour develops during the initial process of hyphal aggregation to form the sheath (Massicotte et al. 1990). The ontogeny of *Pt* ECM formation has been studied in most detail for *Eucalyptus* spp. Using the growth pouch or paper sandwich synthesis systems, ECM formation on first-order lateral roots occurs within 10 days of inoculation (Massicotte et al. 1987b; Horan et al. 1988). ECM formation can be initiated by either diffuse or rhizomorphic hyphae and will occur on lateral roots as they develop or after initial development has occurred, the latter becoming infected only in the apical portion and roots taking on a match-like appearance (Massicotte et al. 1987a, b). Studies of the early stages of *Pt* ECM formation on *Eucalyptus* indicate that sheath formation can commence within approximately 2 days of inoculation, being more rapid at the apex; during the subsequent 2 days Hartig net formation may be initiated (Horan et al. 1988; Lei et al. 1990a,b). Colonisation of *Pinus* roots by *Pt* occurs within a similar timeframe (Piché and Peterson 1988). *Pt* hyphae may penetrate moribund root cap cells during the early days of eucalypt root colonisation; however, intercellular penetration in Hartig net establishment is confined to epidermal cells formed subsequent to fungal colonisation (Horan et al. 1988). During initial contact with the host surface, *Pt* hyphae undergo a morphogenetic shift and produce repeated apical branchings that result in a labyrinthine growth form on the host surface (Jacobs et al. 1989). Root hairs that developed prior to colonisation appear to collapse (Massicotte et al. 1987b; Thomson et al. 1989) but root hairs developing during infection (within the zone of Hartig net development) may become ensheathed by *Pt* (Thomson et al. 1989; Regvar and Gogola 1996). Root hairs colonised in this way clearly cease to function in a normal fashion and appear to degenerate as the ECM matures (Thomson et al. 1989). There is evidence that *Pt* can stimulate host plant ethylene production during early ECM formation and it has been suggested that this may be important in ECM morphogenesis (Rupp et al. 1989). Ethylene production is assumed to be triggered by the production of IAA by *Pt* (Rupp et al. 1989), there being evidence for production of IAA and other indolic compounds by *Pt* in culture (Frankenberger and Poth 1987; Ho 1987; Gruhn et al. 1992; Beguiristain et al. 1995). Host plant jasmonic acid has also been suggested to play a role in Pt infection (Regvar and Gogala 1996), although there is little indication of a potential role for jasmonate at present.

In longitudinal section, the *E. pilularis* – Pt ECM comprises a pre-Hartig net zone, Hartig net zone and older Hartig net zone, which are sequentially formed proximal to the root tip (Massicotte et al. 1987b). Although all three regions of the mycorrhiza possess a thick, compact mantle, there is no Hartig net development in the pre-Hartig net zone, while in the older Hartig net zone, there is evidence of host tissue degeneration and penetration of epidermal cells by the fungus (Massicotte et al. 1987b). Penetration of host cortical cell walls also occurs in the senescent stages of *Pt* – *Pinus* ECM (Nylund et al. 1982). The Hartig net is narrow and penetrates only to the root exodermis in angiosperms. During Hartig net formation, *Pt* continues to branch extensively to produce the labyrinthine structure that typifies the Hartig net in many ECM fungi (Massicotte et al. 1987a,b,c, 1990). Such hyphae frequently contain large lipid bodies (Massicotte et al. 1987a). In conifer hosts, the Hartig net produced by *Pt* generally penetrates 2–3 layers of cortical cells (Molina and Trappe 1982b). The extent to which fungal extracellular enzymes (as opposed to mechanical forces) are involved in penetration of *Pt* hyphae between cortical cells during Hartig net formation remains unclear; however, the shape and small size of some penetration points, as seen in scanning electron micrographs of *Pinus taeda* roots, has been taken to imply that enzymic digestion is involved in some cases (Warrington et al. 1981). It is noteworthy also that penetration of host cells by the fungus during the early and advanced

stages implies an ability to produce wall-degrading enzymes (see below). No evidence exists that the host wall proliferates in response to *Pt* infection (Massicotte et al. 1987a,b,c, 1990), the surface area at the interface between the symbionts being maximised simply by labyrinthine growth of *Pt* and marked radial enlargement of host epidermal cells in response to infection (Massicotte et al. 1987b). The recent observation that a single isolate of *Pt* forms a typical sheath and Hartig net (along with radially enlarged host epidermal cells) in *Quercus acutissima* but only a sheath in *Q. serrata* (Oh et al. 1995) strongly suggests a degree of host control over the nature of the exchange interface that develops with *Pt*. It may, thus, represent an excellent model system for more detailed investigation of aspects of the *Pt* – host interaction.

Pt can increase root branching to form second- and third-order laterals in both conifer and angiosperm hosts (Sohn 1981; Wullschleger and Reid 1990; Oh et al. 1995). There is also evidence that ECM formation by the fungus results in increased lateral root length in a conifer host (Regvar and Gogala 1996). In *E. pilularis* inoculated with *Pt*, first-order mycorrhizal laterals give rise to second-order laterals acropetally along the first-order roots. Second-order laterals in turn yield third-order mycorrhizal laterals, leading to the formation of *Pt* mycorrhizal clusters (Massicotte et al. 1987b). Second-order laterals are produced in the mature region of the Hartig net zone; however, the Hartig net does not spread internally from the parent root, and developing laterals are infected by inward growth of surface hyphae (Massicotte et al. 1987b).

Symbiotic functioning

In compatible *Pt* – *Eucalyptus* ECM at least, the fungal sheath surrounding short lateral roots forms a selectively permeable barrier between the root surface and the soil solution (Ashford et al. 1989). Specifically, carbohydrate deposited in the interhyphal spaces of the sheath appears to be of low permeability to solutes, preventing movement through the sheath apoplast. In this way, the fungus – root interface in the *Pt* – *Eucalyptus* ECM has been described as a sealed apoplastic compartment bounded by the impermeable sheath on one side and the root exodermis on the other, within which the physico-chemical environment (and so presumably nutrient exchange) can be controlled by the two partners (Ashford et al. 1989).

The discovery of a motile vacuolar system within *Pt* hyphae which extends through the dolipore septa and can act as a vehicle for inter-cell transport has provided fresh insight into mechanisms of translocation in fungi (Shepherd et al. 1993a,b; Orlovich and Ashford 1994). The vacuolar system within an individual *Pt* hypha can display spatial variation in internal pH, suggesting heterogeneity and functional diversity within the interconnected system (Rost et al. 1995). Equally, whereas polyphosphate accumulation in vacuoles of *Pt* has previously been described as being insoluble and/or granular in nature (eg. Ashford et al. 1986; Orlovich et al. 1989, 1990), recent data suggest that polyphosphate granules reflect an artefact of specimen preparation and that polyphosphates in vacuoles of *Pt* are in fact soluble (Orlovich and Ashford 1993; Ashford et al. 1994). Taken together, these data implicate translocation of soluble polyphosphate along *Pt* hyphae as the major means of phosphorus transport from the soil solution to the fungus–root interface.

Both absorption and efflux of phosphorus from *Pt* mycelia appear to be governed strongly by the intracellular (presumed to be vacuolar) inorganic phosphate (P_i) concentration; absorption being maximal at low intracellular P_i concentrations, with net efflux occurring under conditions where high intracellular P_i concentrations are predicted (Cairney and Smith 1992, 1993). As recently suggested by Cairney and Burke (1996), differential expression of polyphosphate kinase and polyphosphatase activities in the extramatrical mycelium and in hyphae at the fungus – root interface, respectively, might maximise the efficiency of absorption from soil and transfer to the host root at the exchange interface (by regulation of P_i versus polyphosphate concentrations). Although intracellular polyphosphatase activities are known to be produced by *Pt* (Tillard et al. 1989), differential expression in spatially separated regions of an individual mycelium has yet to be demonstrated.

Host plant growth responses and fungus-derived benefits

Growth responses

Growth responses following seedling inoculation under controlled conditions with *Pt* (both gymnosperm and angiosperm hosts) have been repeatedly observed (eg. Marx and Bryan 1970; Beckjord et al. 1985; Heinrich et al. 1988; Bougher and Malajczuk 1990; Burgess et al. 1994). Growth responses of *Eucalyptus* and *Pinus* spp. to inoculation with *Pt* are, however, strongly influenced by fungal genotype (Dixon et al. 1987; Lamhamedi et al. 1990; Burgess et al. 1994; Thomson et al. 1994). Growth stimulation in *E. grandis*, for example, varied from 2–45 times that of controls in a single study using 20 *Pt* genotypes, the extent of the growth response being correlated with the degree of mycorrhization (Burgess et al. 1994). It is noteworthy that in some instances, *Pt* infection under controlled conditions has been associated with significantly reduced host growth, particularly under semi-hydroponic conditions where nutrient depletion zones do not occur (Tonkin et al. 1989; Eltrop and Marschner 1996a).

Edaphic factors can also influence host growth responses under controlled conditions. Although *Pt* displays intraspecific variation with respect to temperature

optima for growth in axenic culture and mycorrhization, root colonisation under controlled conditions is generally better at relatively high temperatures (above about 19 °C) (Marx and Davey 1969a; Marx et al. 1970; Cline et al. 1987), and growth responses of *Pinus* spp. can be enhanced at such temperatures (Marx and Bryan 1971). Under relatively cool conditions, slow growth and consequent poor infection of newly forming short lateral roots of *E. diversicolor* by *Pt* has also been shown to preclude a growth response (Bougher et al. 1990). Increasing soil moisture content or nutrient status (particularly phosphorus) can similarly reduce infection levels and *Pt*–induced growth responses (Marx et al. 1982; Beckjord et al. 1985; Bougher and Malajczuk 1990). In the case of nitrogen, NO_3^- has been shown to decrease *Pt* infection in *Picea abies*, although the extent to which this reflects the poor ability of *Pt* to utilise NO_3^- as a nitrogen source or some indirect effect on the symbiotic interaction remains to be determined (Eltrop and Marschner 1996a). Atmospheric gas composition can also influence rates of ECM formation by *Pt*. While elevated atmospheric CO_2 concentrations can increase colonisation, extramatrical mycelium production and host growth (O'Neill et al. 1987; Ineichen et al. 1995; Walker et al. 1995), NO_x has no apparent effect (Näsholm et al. 1991). Conversely, increasing ozone concentration or soil acidification can result in decreased infection (Adams and O'Neill 1991; McQuattie and Schier 1992; Maehara et al. 1993), although the latter has not been recorded in all cases (Mahoney et al. 1985; Keane and Manning 1988).

Aggregate data from a number of field studies indicates that, although responses are variable, specific isolates of *Pt* can enhance tree growth, particularly under relatively warm, dry conditions (eg. Roland and Albaladejo 1994). The degree of root colonisation by *Pt* at outplanting appears to be a good indicator of subsequent field performance, especially in drier years (Marx and Hatchell 1986). In temperate regions, growth stimulation in *Pt*–infected plants over controls (infected by indigenous fungi) have been reported to extend for up to 7 years in *Pinus* spp. outplanted in sandy soils and mine spoils in southeastern USA (Hatchell and Marx 1987; Walker et al. 1989). This effect was species specific, with *P. taeda*, for example, showing a *Pt*–induced growth stimulation for only a single growing season. Clearly such growth responses are also site-specific, as *Pt* stimulated growth of *P. taeda* for over 8 years in another study (Marx and Cordell 1988). Outplanting trials in the tropics indicate that growth responses in *Eucalyptus* spp. and *Pinus* spp. can be obtained in the short term following *Pt* inoculation. The degree of growth enhancement is again variable, but in general appears more marked at drier sites with high soil temperatures and in times of lower precipitation (Momoh and Gbadegesign 1980; Marx et al. 1985; Le Tacon et al. 1988). The rapid and prolific sporulation of *Pt* may also be of benefit at some tropical sites in promoting infection and so enhancing growth of neighbouring, previously uninfected plantation pines (Le Tacon et al. 1988). In contrast, outplanting trials with conifers in cooler parts of the USA indicate that *Pt* provides no overall growth stimulation when compared to seedlings infected by indigenous fungi (Danielson and Visser 1989; Castellano and Trappe 1991). It is thus regarded as being of little value in boreal forestry (Navratil et al. 1981).

The lack of sustained growth enhancement in outplanted seedlings under cool conditions is widely held to reflect the relatively poor ability of *Pt* to compete with indigenous ECM fungi in plantation soils (Marx et al. 1984). Thus complete replacement of inoculated *Pt* on conifer roots by indigenous mycobionts has been reported within 3–5 years following outplanting (Riffle and Tinus 1982; Grossnickle and Reid 1983; Danielson and Visser 1989). Replacement of inoculated *Pt* can be much more rapid than these data suggest. ECM fungal communities on outplanted seedlings monitored over a shorter time frame have shown a complete loss of *Pt* within 2 months of outplanting at some sites (McAfee and Fortin 1986). Persistence of *Pt* appears to be greatest in soils more similar to the natural habitats of *Pt* and in situations, such as on extreme acid mine sites, where edaphic stresses result in less intense competition from other ECM mycobionts (Schramm 1966; Berry 1982; McAfee and Fortin 1986, 1988). The general soil microflora may also influence mycorrhiza formation and persistence of Pt, with enhancement or depression depending on the microflora composition (Bowen and Theodorou 1979; Aggangan et al. 1996).

Nutritional benefits to the host

Phosphorus

Growth enhancement of conifers and eucalypts associated with *Pt* infection has frequently been correlated with increased phosphorus accumulation in the host (eg. Heinrich et al. 1988; Rousseau et al. 1992; Burgess et al. 1993; Thomson et al. 1994), although this is not always the case (Walker et al. 1989). Enhanced phosphorus accumulation appears to relate to the level of mycorrhizal infection and the surface area of the extramatrical mycelial phase (Rousseau and Reid 1990; Rousseau et al. 1992, 1994; Thomson et al. 1994). There is direct evidence that *Pt* can absorb orthophosphate from solution in the external environment and transfer absorbed phosphorus to the host following translocation through extramatrical mycelium (Kammerbauer et al. 1989). *Pt* has been shown to solubilise relatively insoluble forms of inorganic phosphate (Al and Ca phosphates) *in vitro*, although this ability appears to be isolate specific (Lapeyrie et al. 1991). Further, it has been shown that infection with *Pt* can increase host access to insoluble inorganic phosphate sources, such as Fe/Al PO_4, in *Eucalyptus pilularis* (Heinrich et al. 1988). As is the case for most ECM basidiomycetes, isolates of *Pt* can utilise soluble salts of inositol hexaphosphate as

sole phosphorus source (Mousain and Salsac 1986). Although interspecific variation exists in relative importance, utilisation appears to be based upon production of extracellular acid and alkaline phosphomonoesterase and phosphodiesterase enzymes (Ho 1987). Acid phosphomonoesterase production is stimulated by deficiency of inorganic phosphate (Berjaud and d'Auzac 1986; Mousain and Salsac 1986). While it is generally assumed to occur, transfer to the host plant of phosphorus derived from organic phosphate sources remains to be demonstrated for *Pt*.

Nitrogen

Enhanced seedling growth in response to *Pt* inoculation can in some instances be correlated with increased foliar nitrogen content (Wullschleger and Reid 1990). Since foliar nitrogen concentration has in turn been positively correlated with endogenous plant cytokinin levels, it has been suggested that nitrogen may act via cytokinins as a metabolic regulator (Wullschleger and Reid 1990). *Pt* mycelia growing in axenic culture and conifer roots infected by the fungus can absorb inorganic nitrogen as either NH_4^+ or NO_3^-, although absorption rates are greater for NH_4^+ than for NO_3^- (France and Reid 1983, 1984; Eltrop and Marschner 1996a). *Pt* has been shown to transfer N absorbed in the form of NH_4^+ to *Pinus sylvestris* host plants (Finlay et al. 1988). As with several other ECM fungi, absorbed NH_4^+ appears to be incorporated rapidly into amino acid precursors within the extramatrical mycelium and translocated to the host largely in this form (Finlay et al. 1988). While an enzymological study of NH_4^+ metabolising enzymes has suggested that *Pt* produces only low levels of glutamine synthetase (GS) and no glutamate synthase (GOGAT) activity (Vézina et al. 1989), studies of $^{15}NH_4^+$ metabolism provide strong evidence that NH_4^+ is metabolised via the GS/GOGAT pathways (Kershaw and Stewart 1992; Turnbull et al. 1996). This apparent discrepancy may reflect that single (and different) isolates of *Pt* were used in each study, and that intraspecific differences in inorganic N assimilation exist. More likely, however, they reflect the relatively low sensitivity of enzyme assays compared with direct assessment of ^{15}N assimilation using a mass spectrometer.

When grown in axenic culture, *Pt* can release and utilise NH_4^+ (and also Ca_2^+) ionically bound to vermiculite, suggesting a potential to partially weather phyllosilicates in soil (Paris et al. 1995b). While soluble fungal exudates appear to be responsible for such weathering, it remains to be determined to what extent the host benefits from this potential nitrogen source. Data on the ability of *Pt* to enhance host plant acquisition of nitrogen from organic sources are rather more equivocal. *Pt* has been classified as a 'non-protein' fungus based on the relatively poor ability of certain isolates to produce extracellular protease activity in axenic culture (Abuzinadah and Read 1986; Cao and Crawford 1993a). This notwithstanding, it is clear that some *Pt* isolates secrete protease under some conditions (Dahm and Strzelczyk 1995). Similarly, while Abuzinadah et al. (1986) concluded that *Pt* only poorly enhanced acquisition of nitrogen from protein by *Pinus contorta*, Turnbull et al. (1995) recently showed enhanced utilisation of nitrogen in protein and histidine sources by *Eucalyptus* spp. in symbiosis with *Pt*. Such disparate results may indicate the influence of different host plants, perhaps mediated by differential availability of carbon compounds from each host, as suggested by Turnbull et al. (1995). Equally, since in each case only single isolates of *Pt* were utilised, there may simply be considerable intraspecific variation within *Pt* with regard to facilitating organic N utilisation. Recent results from our laboratory indeed indicate wide intraspecific variation in *Pt* in this respect (JM Sharples and JWG Cairney, unpublished data).

Mineral transformations

Pt can bring about several mineral transformations which may be important in increasing the availability of certain elements in soil. In axenic culture, isolates of *Pt* are known to bring about oxidation of elemental sulphur (Grayston and Wainwright 1988) and to produce an extracellular substance capable of reducing higher oxides of manganese (Cairney and Ashford 1991). The latter activity is apparently also produced during symbiosis with the host (Cairney and Ashford 1989) and there is direct evidence that *Pt* can enhance manganese accumulation from soil by *Pinus virginiana* seedlings (Miller and Rudolph 1986). *Pt* has also been shown to displace and render available K^+ from non-exchangeable sites in phlogopite mica, probably via secretion of oxalate (Paris et al. 1995a, 1996). Isolates of *Pt* can produce hydroxamate-like siderophores which may be important in chelating scarcely available soil iron compounds (Szaniszlo et al. 1981; Leyval and Reid 1991). Such chelating compounds can be absorbed by both mycorrhizal and non-mycorrhizal host roots (Leyval and Reid 1991).

Although the fungus is generally assumed to have a poor ability to decompose carbohydrate components of the plant cell wall, recent evidence indicates that components of the cellulase complex are produced differentially by different *Pt* isolates. Thus, β-glucosidase production may be a common feature of many *Pt* isolates, while only particular isolates produce β-galactosidase and/or both endo- and exo-acting glucanases (Cao and Crawford 1993a,b). Production of cellulases during symbiosis with the host has not yet been shown, but it is possible that these enzymes are involved in establishment of the symbiosis (see above), along with hyphal penetration of host walls as the symbiosis ages and possible interactions between extramatrical mycelium and moribund plant material in soil (see Cairney and Burke 1994).

Influence on host plant carbon economy

Infection of conifer seedlings with *Pt* generally results in increased rates of net photosynthesis (Ekwebelam and Reid 1983; Reid et al. 1983; Rousseau and Reid 1990); under some conditions increases over uninfected seedlings are of the order of 75% (Rousseau and Reid 1989). Where soil has a relatively high phosphorus status, *Pt* may have a neutral effect on host photosynthesis and result in poorer host growth compared with uninfected controls (Rousseau and Reid 1989); the increased respiratory cost of the fungus has a negative effect on host carbon balance. Where increased photosynthetic rate has been observed, it has generally been associated with an increase in host plant biomass (Rousseau and Reid 1990; Reid et al. 1983). Increases in net photosynthesis and host biomass show a strong correlation with the degree of root system infection with *Pt* (Rousseau and Reid 1990). By comparing *Pt*–infected seedlings to seedlings fertilised with different levels of phosphate, Rousseau and Reid (1990) concluded that, at low infection rates, increased photosynthesis in *P. taeda* attributable to infection is probably the result of enhanced phosphate accumulation. Where infection rates are high, however, it appears that the increased carbon sink created by the fungus results in increased photosynthesis in a more direct manner (Rousseau and Reid 1990). *Pt* has also been shown to maintain high rates of gas exchange and photosynthesis in *Eucalyptus* sp. during drought stress (Dixon and Hiol-Hiol 1992). In circumstances where *Pt* infection has reportedly reduced host plant growth, infection may still increase rates of CO_2 assimilation in the host (Eltrop and Marschner 1996b). In such circumstances, however, it is likely that increased below-ground respiration resulting from the presence of ECM mycelium imparts a significant drain on host carbon resources in the absence of a significant nutritional benefit.

Clear evidence exists that *Pt* acts as a significant sink for host-derived carbon, at least during the early phase of the symbiosis. Cairney et al. (1989) using $^{14}CO_2$ pulse labelling showed that 18 times more carbon can accumulate in *Pt*–infected *Eucalyptus pilularis* short lateral roots than in uninfected roots in the same root system. These data are likely to underestimate the increased sink created by *Pt* since they take into consideration neither carbon translocated from mycorrhizal tips into extramatrical mycelium nor fungal respiration. These may be significant given the considerable labelling of extramatrical mycelia in the autoradiographs produced by Cairney et al. (1989) and the reported threefold greater root-derived respiration in *Pt*–infected *P. contorta* recorded by Reid et al. (1983). As the *Pt* – eucalypt association ages, there appears to be a progressive decrease in the degree to which it acts as a sink for host photosynthetic products (Cairney et al. 1989). There is also evidence that small quantities of carbon compounds can be transferred between host plants interconnected by a common *Pt* mycelium (Finlay and Read 1986). The main soluble carbohydrate in *Pt* extramatrical mycelia and mycelia grown in axenic culture is arabitol, but trehalose and mannitol have also been shown to be present in significant quantities (Söderström et al. 1988; Ineichen and Wiemken 1992). While the main fungal carbohydrate in *Picea abies* mycorrhizas is trehalose (Ineichen and Wiemken 1992), the preponderance of arabitol in *Pt* mycelia, strongly supports the latter as the major translocatory carbohydrate in *Pt*.

Non-nutritional benefits to the host

Infection of both angiosperm and gymnosperm seedlings with *Pt* can reduce host water deficit under conditions of mild drought (Dixon et al. 1983; Parke et al. 1983; Walker et al. 1989). The ability of *Pt* to ameliorate drought stress appears to be strongly isolate specific, there being a demonstrated correlation between the ability of isolates to produce extensive rhizomorph systems and their ability to enhance host water status (Lamhamedi et al. 1992a,b). While temperature and drought stresses may be important in determining the survival of *Pt* on mine sites, persistence of the fungus also requires a low sensitivity to toxic metals. *Pt* can reduce Zn accumulation in conifer shoots when grown on contaminated coalspoils (Walker et al. 1989) or in *Zn*–supplemented soil in the glasshouse (Miller and Rudolph 1986). Reduced Zn accumulation in the shoot is accompanied by an increase in Zn accumulation in *Pt*–infected versus non-mycorrhizal roots (Miller and Rudolph 1986). The ability of *Pt* to ameliorate Zn sensitivity seems likely to be isolate specific, since in some instances infection results in an increase in foliar Zn (Berry and Marx 1976). Similarly, while a single isolate of *Pt* has been shown recently to be ineffective in ameliorating Pb toxicity in *Picea abies* (Marschner et al. 1996), screening with multiple isolates may reveal less sensitive isolates.

Because of the potential toxicity to forest trees of Al in acid soils, particularly under the influence of acid precipitation, there has been interest in the potential ability of *Pt* to ameliorate the problem. Infection with *Pt* can reduce Al accumulation in the host shoot and can partially alleviate Al sensitivity in *Pinus* (Berry and Marx 1976; Cumming and Weinstein 1990a; Schier and McQuattie 1995). This may apply only up to certain Al concentrations, since mycorrhiza formation by *Pt* can be inhibited at high concentrations (McQuattie and Schier 1992). The precise mechanism of the reduced host sensitivity is not clear, although reduced Al uptake by the host (perhaps due to the diffusion barrier presented by the sheath) is thought to be involved to some extent (Cumming and Weinstein 1990b; Schier and McQuattie 1995; Godbold et al. 1996). Increased host phosphorus status (and so enhanced host vigour), arising from an ability of the fungus to prevent precipitation of $AlPO_4$ in the rhizosphere and root apoplast may

also play a role (Cumming and Weinstein 1990b; Schier and McQuattie, 1995). The preference of *Pt* for NH_4^+ rather than NO_3^- as a nitrogen source might also be involved. Since nitrate reductase is Al sensitive, a switch to the less sensitive NH_4^+ assimilation pathways in *Pt* ECM could be important in ameliorating toxicity (Cumming 1990; Cumming and Weinstein 1990a,b). Godbold et al. (1996) have further suggested that the lower pH of the apoplast arising during NH_4^+ utilisation may also be important in preventing Al accumulation in walls of the host cortex. From a recent study of 21 *Pt* isolates originating from soils of differing Al and pH status, it is clear that considerable intraspecific variation in Al sensitivity in axenic culture exists (Egerton-Warburton and Griffin 1995). The degree of sensitivity was inversely correlated with the relative availability of Al in the soil of origin, although the authors were careful to point out that the number of isolates screened was relatively small, that most of the isolates came from a heavily contaminated site and that a larger number of samples from sites contaminated to a lesser degree would need to be included for a proper correlation to be derived. The differential abilities of these isolates to ameliorate plant sensitivity to Al remains to be investigated.

The mechanisms involved in metal detoxification by *Pt* have not been investigated in detail. However, intracellular metallothionein-like proteins were induced by toxic metal exposure in a single *Pt* isolate (Morselt et al. 1986), while an increase in intracellular tyrosinase activity in another isolate (in response to Cu exposure) was implicated in chelation of intracellular Cu (Gruhn and Miller 1991). Polysaccharides and cysteine-rich proteins were shown to accumulate on the outer cell wall in a further *Pt* isolate in response to extracellular Cd exposure, and electron energy loss spectroscopy (at the TEM level) was used to demonstrate apparent accumulation of Cd on the outer region of hyphal walls (Turnau et al. 1994). While the latter observation suggests a role for the modified cell wall in Cd detoxification, it must be viewed with a degree of caution since material was prepared for electron microscopy using conventional (hydrated) preparative techniques that do not preclude redistribution of ions during specimen preparation. Where ECM have been prepared for electron microscopy/X-ray microanalysis using cryo-methods, Al appears to accumulate specifically in the *Pt* sheath (Egerton-Warburton et al. 1993). In the case of Al detoxification, enhanced Ca and Mg accumulation in tolerant *Pt* isolates may alter the ratio of the divalent cations to Al, reducing binding and absorption of Al and so decreasing toxicity (Egerton-Warburton and Griffin 1995).

Pt may also be of value in remediating sites contaminated by xenobiotic organic chemicals. Although only two investigations of the fungus in axenic culture have been conducted to date, Donnelly and Fletcher (1995) have identified an isolate of *Pt* that possesses some ability to degrade polychlorinated biphenyls, while Meharg et al. (1997) indicate an ability to biotransform 2,4,6-trinitrotoluene. Further screening of *Pt* growing in symbiosis with a host is required to determine its real potential in remediation of sites contaminated by organic pollutants.

It has been known for some considerable time that *Pt* has the potential to protect seedlings against a variety of soil-borne pathogens (see Marx 1972). For example, *Pt* has been reported to confer a degree of protection to *Pinus* spp. against (among others) *Phytophthora*, *Fusarium*, *Rhizoctonia* and *Cylindrocarpon* spp. in glasshouse trials (Ross and Marx 1972; Chakravarty and Unestam 1987a,b). In some instances, the protective effect of *Pt* isolates was attributed solely to the provision of a physical barrier by the fungal sheath, with no evidence of production of antimicrobial activities by the fungus (Marx and Davey 1969b; Marx 1970). There have, however, been many reports of *in vitro* inhibition of the growth of a range of pathogens in the presence of *Pt* mycelium, strongly implying production of antimicrobial metabolites (eg. Marx 1969; Kope and Fortin 1989a,c), although the degree of this effect is isolate specific (Kope and Fortin 1989b; Suh et al. 1991). Unidentified phenolic compounds (Suh et al. 1991) were implicated in one study, while two specific antifungal compounds [*p*–hydroxybenzoylformic acid (pisolithin A) and (R)-(–)-*p*–hydroxymandelic acid (pisolithin B)] were isolated by other workers and their effectiveness demonstrated against a range of pathogens in vitro (Kope and Fortin 1989a; Kope et al. 1991). Production of these secondary metabolic products has not yet been confirmed during symbiosis with a host. However, given the physiological heterogeneity within individual ECM mycelia, and the potential for idiophase (secondary metabolism) onset in different spatio-temporal regions therein (see Cairney and Burke 1994, 1996), it is certainly conceivable that such products can be expressed in symbiotic *Pt* mycelia in soil. It is further possible that the extracellular antimicrobial effect of *Pt* is enhanced by non-specific acidification of the rhizosphere (Rasanayagam and Jeffries 1992).

Conclusions

A considerable body of literature exists on the ecology, physiology and molecular biology of interactions between *Pt* and its plant hosts. From work conducted to date, it is clear that considerable intraspecific variation exists within *Pt* in host specificity, growth form of extramatrical mycelia and organic nitrogen utilisation. Significant progress is currently being made towards an understanding of the infection process and compatibility between *Pt* and various hosts using multiple *Pt* isolates, although the extent to which differential host compatibility reflects taxonomic variation within the *Pt* group is not yet clear. It may be, however, that careful examination of phylogeny within *Pt* at the molecular level will reveal a genetic basis for differential host spe-

cificity. In many instances, physiological aspects of the fungus – host interaction have been studied simply in the form of observations of individual *Pt* mycelia or comparisons between single *Pt* isolates and other ECM fungi. Given the level of variation displayed within *Pt* in other aspects of the symbiosis, such data should be used with caution to extrapolate the physiological capabilities of the fungus. Physiological screening of a range of *Pt* isolates, preferably those isolates upon which current molecular and host compatability investigation is focussed, will be required in order to develop a true picture of *Pt* ECM symbioses and their effectiveness in enhancing host plant growth and survival.

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