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The postfire discomycete *Geopyxis carbonaria* (Ascomycota) is a biotrophic root associate with Norway spruce (*Picea abies*) in nature

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Abstract

The hypothesis that the postfire discomycete *Geopyxis carbonaria* (Ascomycota, Pezizales, Pyronemataceae) has a biotrophic association with roots of Norway spruce (*Picea abies*) in nature was tested by isolation of fungal strains from fresh, brown, smooth mycorrhiza-like root tips of Norway spruce collected from below the depth of detrimental heat penetration in a postfire site. The morphology of seven culture isolates originating from the smooth mycorrhiza-like root tips of two different spruce trees was congruent with the morphology of axenic culture isolates obtained from ascospores of *G. carbonaria*. DNA sequences of the nuclear ribosomal internal transcribed spacers ITS1 and ITS2 from these root-derived cultures and the ascosporic *G. carbonaria* culture isolates were found to be identical, further supporting the conclusion that the isolates were conspecific. The extensive ascocarp and ascospore formation of *G. carbonaria* which succeeds a forest fire may be explained in terms of a fungal escape from a moribund tree associate. Possible ecological adaptations of *G. carbonaria* to the pre- and postfire community are discussed.

Keywords: disturbance, fire ecology, life history, molecular identification, mycorrhiza, species-specific DNA

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Introduction

A group of fungal species within the discomycete order Pezizales is commonly observed after forest fires (Petersen 1970). Their occurrence has been explained in a variety of ways including mycostasis (Wicklow & Zak 1979), tolerance to chemical products of burning (Widden & Parkinson 1975), response to environmental volatiles (Moore-Landecker 1988), reduced competition (El-Abyad & Webster 1968b; Wicklow & Hirschfield 1979), and adaptations to the physicochemical properties of the postfire habitat, e.g. high optimum growth temperatures, high pH tolerance, and tolerance to reduced moisture-holding capacity of the postfire substrate (El-Abyad & Webster 1968a; Petersen 1970).

Geopyxis carbonaria (Alb. & Schw.: Fr.) Sacc. (Pyronemataceae, Pezizales) is one of the most abundant postfire discomycetes in boreal forests (Moser 1949; Petersen 1970; Turnau 1984; Holm 1995). Petersen (1970) observed that *G. carbonaria* produced ascocarps from 16 to 139 weeks after a forest fire. He also observed that the production of ascocarps was consistently associated with the presence of coniferous trees, and suggested that the fungus was involved in the postfire decomposition of coniferous roots and litter. Egger (1986), and Egger & Paden (1986a,b) demonstrated that *G. carbonaria* produces phenol oxidase and degrades lignin, causes weak symptoms of pathogenic infection on seeds and germinants of lodgepole pine (*Pinus contorta*), and forms a mantel and a rudimentary Hartig net with extensive inter- and intracellular penetration of epidermal and cortical cells of roots of lodgepole pine *in vitro*. It was suggested that *G. cabonaria* exhibited both decomposing and biotrophic potentials, and might be adapted to infect moribund roots under certain conditions (Egger 1986; Egger & Paden 1986b).

Based on the prevalence of ascocarps of *G. carbonaria* on fire sites of Norway spruce (*Picea abies*) (Petersen 1970; Holm 1995), we hypothesized that *G. carbonaria* is a biotrophic associate of roots of Norway spruce in nature. This hypothesis was tested by comparative studies of axenic culture isolates from mycorrhiza-like root tips of

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Norway spruce and from ascospores of *G. carbonaria*, using both culture morphology and DNA sequencing. We chose to sequence the nuclear ribosomal internal transcribed spacers ITS1 and ITS2 which are known to provide species-specific genotypes in many fungi, including the discomycetes (Egger 1996; Norman & Egger 1996; Holst-Jensen *et al*. 1997).

Materials and methods

Study area and source of material

On June 26th 1992, a forest fire broke out in a mixed coniferous forest in Maridalen, outside Oslo, Norway. The burn constituted 375 da of mixed Norway spruce and Scottish pine (*Pinus sylvestris*) forests and clear-felled 'stubbed' areas of former spruce and pine stands. The burned area was divided into 10 stands based upon forest type, and age and management of the forest prior to the burning (Holm 1995). A brief characterization of the stands is given in Table 1. Ascocarps of *Geopyxis carbonaria* from different stands constituting 24 collections were sampled in the period between April and September 1993. For further analysis rootlets were collected from below the depth of detrimental heat penetration of the fire (cf. Fig. 1C). Rootlet samples were collected from five different, recently windfallen 100-year-old trees of Norway spruce of stand 4, which still supported extensive amounts of fresh brown smooth mycorrhiza-like root tips.

In order to assess the level of intraspecific molecular variation in *G. carbonaria*, we also included three samples of *G. carbonaria* from two separate areas of Norway and from Poland. In addition, material of the closely related taxa *G. rehmii* Turnau and *G.* cf. *vulcanalis* (Peck) Sacc. were included. Table 2 lists the sources and origins of the specimens used in the study.

Isolation and examination of axenic cultures

Mass ascospore discharges from fresh apothecia of *G. carbonaria* were collected onto 1.5% water agar (WA). Germinating ascospores were transferred to and maintained on 2% potato dextrose agar (PDA; Difco Laboratories, Detroit, Michigan, USA) and 10× diluted potato carrot agar (10% PCA; 2 g of potatoes; 2 g of carrots; 1 L of gdH_2O; autoclaved for 10 min; filtered; volume adjusted to 1 L; 15 g of agar added; autoclaved for 20 min). One sample of brown smooth mycorrhiza-like morphotypes from each of the five windfallen trees was examined under a stereo magnifier, and evaluation of viability of the root tips was based on colour and turgescence (Harvey *et al*. 1976). From each of the five samples, 10 viable, light brown and elastic mycorrhiza-like root tips, which may be referable to some of the '*Piceirhiza*' morphotypes of Agerer's (1987–97), were selected and cut into short, 2–5 mm segments. The root-tip segments were surface sterilized in 70% ethanol (20 s) and household bleach (1 min), and finally rinsed twice in autoclaved gdH_2O (Stoyke & Currah 1991). The root tips were transferred onto WA plates containing antibiotics (streptomycin and tetracycline), and incubated in darkness at 20 °C. After 48 h, colonizing mycelium emanating from the root tips was reisolated and transferred onto

Table 1 A brief characterization of the 10 stands in the fire-site of Turtermarka, Maridalen, Oslo, Norway (modified after Holm 1995)

Stand	Forest type	Forest management	Fire type and intensity	Host injury
1	Blueberry-rich spruce forest	Young forest	Ground and crown fire, heavily burnt	Spruce trees severely injured, died within a year after the fire
2	Blueberry-rich spruce forest	Clear felled area	Ground fire, heavily burnt	–
3	Lichen and *Erica*-rich pine forest	Young forest	Surface fire, moderately burnt	Pine trees lightly to moderately injured, all remained viable
4	Blueberry-rich spruce forest	Old forest	Ground and crown-fire, heavily burnt	Spruce trees severely injured, died within a year after the fire
5	Blueberry-rich spruce forest	Clear felled area	Ground fire, heavily burnt	–
6	Lichen and *Erica*-rich pine forest	Old forest	Surface fire, moderately to heavily burnt	Pine trees moderately to severely injured, died within two years after the fire
7	Blueberry-rich spruce forest	Young forest	Surface fire, lightly burnt	Spruce trees lightly injured, all remained viable
8	Lichen and *Erica*-rich pine forest	Clear felled area	Surface fire, moderately burnt	–
9	Blueberry-rich spruce forest	Young forest	Surface fire, lightly burnt	Spruce trees lightly injured, all remained viable
10	Lichen and *Erica*-rich pine forest	Young forest	Surface fire, lightly burnt	Pine trees lightly injured, all remained viable

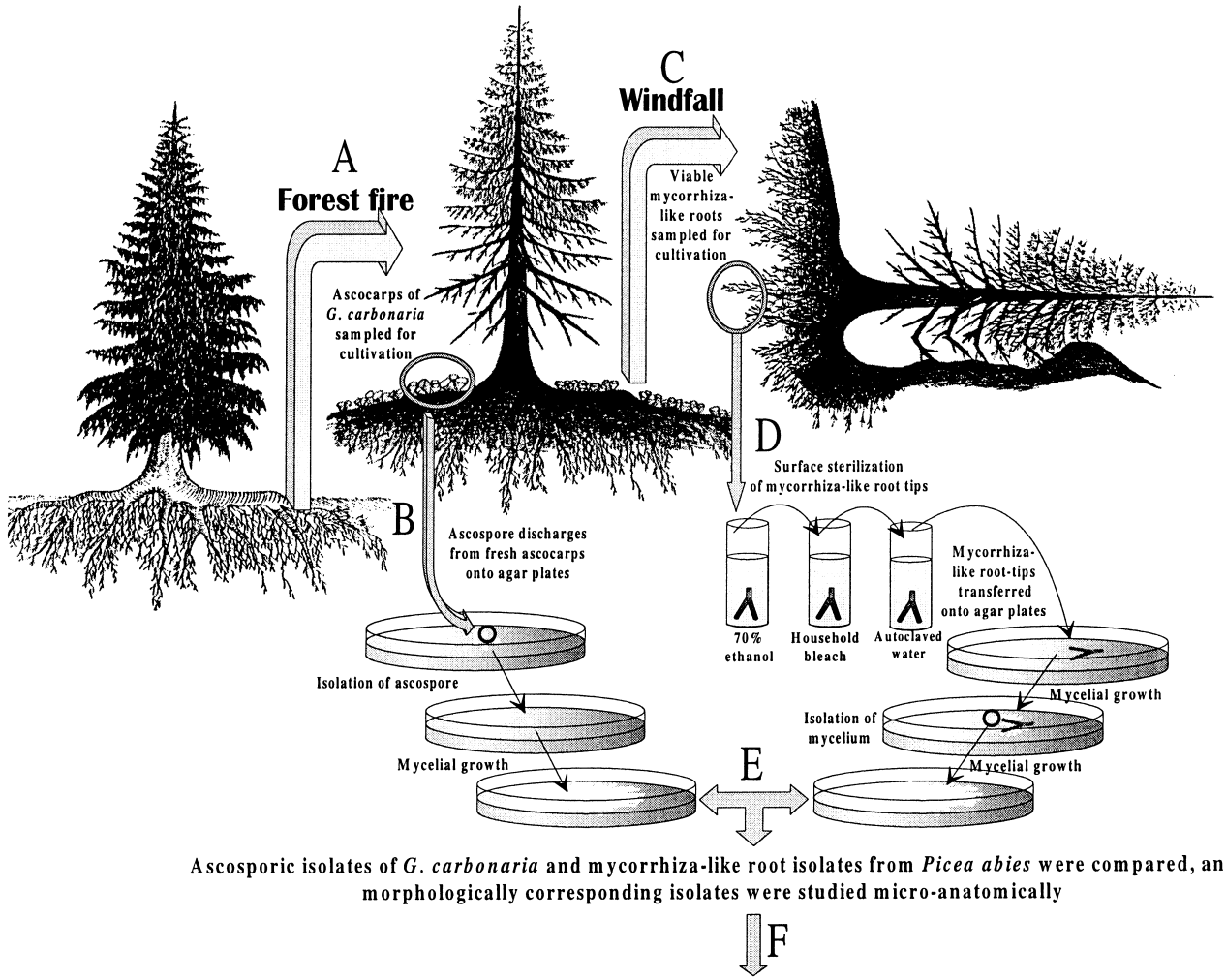

Fig. 1 Schematic presentation of the study protocol. A. After a forest fire extensive production of ascocarps of *Geopyxis carbonaria* was observed. B. Ascocarps were sampled, ascospores were collected onto water agar, and single spores were isolated and grown in axenic culture on nutrient media (PDA and 10% PCA). C. After a heavy storm, several burnt trees were windfelled, and unaffected, still viable mycorrhiza-like roots from below the depth of detrimental heat penetration were exposed. D. Rootlets were sampled from five windfalls, and 50 viable brown, smooth mycorrhiza-like root tips were surface sterilized, and grown in axenic culture. E. Axenic culture isolates from ascospores and root tips were morphologically compared. F. Corresponding ascospore and root isolates were prepared for DNA extraction, PCR amplified, and DNA sequenced. The nuclear ribosomal ITS1, ITS2, and the 5.8S rDNA sequences of the root isolates were compared with sequences from ascospore isolates.

sterile nutrient media (PDA; 10% PCA). The root isolates were reisolated twice. Growth rate and morphology of the root isolates and the ascospore isolates of *G. carbonaria* were compared. Mycelial growth rates were measured on PDA after 1 week and after 3–4 weeks. Micro-anatomical investigations were carried out on fresh culture isolates. Hyphae and anamorphic structures induced on 10% PCA were studied in light microscope from squash mounts in gdH_2O and cotton blue. The root isolates having cultural features similar to those of the strains of *G. carbonaria* were subjected to further analysis. All axenic culture isolates are deposited in the culture collection of the Ascomycete Research group of Oslo, Norway (ARON).

Molecular studies

Table 2 lists specimens submitted to DNA analysis. Axenic culture mycelium was grown on liquid complete yeast medium (CYM), and DNA was extracted as described by Holst-Jensen *et al.* (1997). Nuclear ribosomal ITS1, ITS2, and the 5.8S rDNA was PCR amplified and sequenced as

Table 2 Sequenced isolates of *Geopyxis carbonaria*, *G. rehmii*, *G.* cf. *vulcanalis*, and selected root isolates

Taxon specimen	Isolate* and source†	Origin	Accession no.‡
G. carbonaria	1619.2	Norway, Oslo, Maridalen, fire-site, stand 1	Z96980
G. carbonaria	1685.1	Norway, Oslo, Maridalen, fire-site, stand 1	Z96981
G. carbonaria	1764.1	Norway, Telemark, Bamble, fire-site	Z96982
G. carbonaria	1796.2	Norway, Oslo, Maridalen, fire-site, stand 4	Z96983
G. carbonaria	1754.1	Norway, Hordaland, Sveio, fire-site	Z96984
G. carbonaria	1741.1	Norway, Oslo, Maridalen, fire-site, stand 4	Z96985
G. carbonaria	1797.1	Norway, Oslo, Maridalen, fire-site, stand 4	Z96986
G. carbonaria	1803.1	Norway, Oslo, Maridalen, fire-site, stand 6	Z96987
G. carbonaria	2217.H	Poland, Turbacz, fire-site	Z96988
Root isolate 1	1941.M	Norway, Oslo, Maridalen, fire-site, stand 4	Z96989
Root isolate 2	1942.M	Norway, Oslo, Maridalen, fire-site, stand 4	Z96990
G. cf. *vulcanalis*	2156.H	Norway, Akershus, unburned substrate	Z96992
G. rehmii	2216.H	Poland, Turbacz, fire-site	Z96991

*Each isolate is part of a collection, referring to a sheet of additional information in the database of the Ascomycete Research group of Oslo, Norway (ARON).
†Isolate refers to a voucher specimen or an axenic culture isolate in the culture collection of ARON. A digit after the full stop indicates monospore culture; M indicates culture isolated from mycorrhiza-like root tips, and H indicates an isolate from which no culture has been obtained, from which DNA was extracted from an air-dried herbarium specimen.
‡EMBL/GenBank/DDBJ accession numbers for the ITS sequences including the 5.8S rRNA gene.

described by Holst-Jensen *et al*. (1997) using the primers ITS1, ITS2, ITS3 and ITS4 (White *et al*. 1990). The DNA sequences were manually aligned in SeqApp (Gilbert 1993). Figure 1 provides a visualization of the protocol.

Results

Field observations

Ascocarps of *Geopyxis carbonaria* were abundant during the first year in fire-injured, heavily burnt forested stands (stands 1, 4, and 6, cf. Table 1). Ascocarps were neither observed in the lightly to moderately burnt stands where trees remained viable (stands 3, 7, 9 and 10), nor in the clear-felled areas (stands 2, 5, and 8). During the vegetation period from April to September 1993, the spruce stands (stands 1 and 4) exhibited a much higher density of ascocarps of *G. carbonaria* than the pine stand (stand 6). Frequently as many as 700–1000 ascocarps/m^2 of the taxon were observed on the spruce postfire forest floor, in contrast to few, small and scattered clusters of ascocarps on the pine forest floor.

ITS sequence variation of the Geopyxis *species*

The sequence alignment is shown in Fig. 2. The ITS sequences of the specimens of *Geopyxis carbonaria* constituted 512 bp of the alignment (ITS1 = 193 bp; 5.8S = 158 bp; ITS2 = 161 bp), and only three substitutions were observed (positions 35, 36, 38 of the alignment, all within the ITS1). The ITS sequences of *Geopyxis rehmii* and *Geopyxis* cf. *vulcanalis* constituted 546 bp (ITS1 = 227 bp; 5.8S = 158 bp; ITS2 = 161 bp) and 573 bp (ITS1 = 254 bp; 5.8S = 158 bp; ITS2 = 161 bp) of the alignment, respectively. The ITS sequence divergence between *G. carbonaria*, *G. rehmii* and *G.* cf. *vulcanalis* consisted of one large (positions 29–95 of the alignment), and two small (positions 103–104 and 135 of the alignment) insertions/deletions (indels) within the ITS1, and several substitutions within the ITS1 and ITS2. The 5.8S rRNA gene yielded no interspecific sequence variation.

Morphological and molecular comparison of culture isolates from ascospores and roots

Isolation of fungal strains from 50 brown, smooth mycorrhiza-like root tips of Norway spruce yielded a total of 35 axenic culture isolates which were roughly divided into five groups based on growth rates and macromorphology. One group, consisting of seven isolates from two different windfall samples, corresponded in morphology to the ascospore isolates of *G. carbonaria* in all cultural characteristics. On PDA, both the ascosporic isolates of *G. carbonaria* and the similar root isolates exhibited radial, even and compact growth, the mycelial mats reaching a diameter of 2–3 cm in one week and a maximum of ≈ 6–7 cm in diameter after 3–4 weeks. The mycelial mats were covered by persistently white and cottony mycelium, underneath consisting of a leathery to cartilaginous mat of densely interwoven hyphae. On 10% PCA the diameter of the

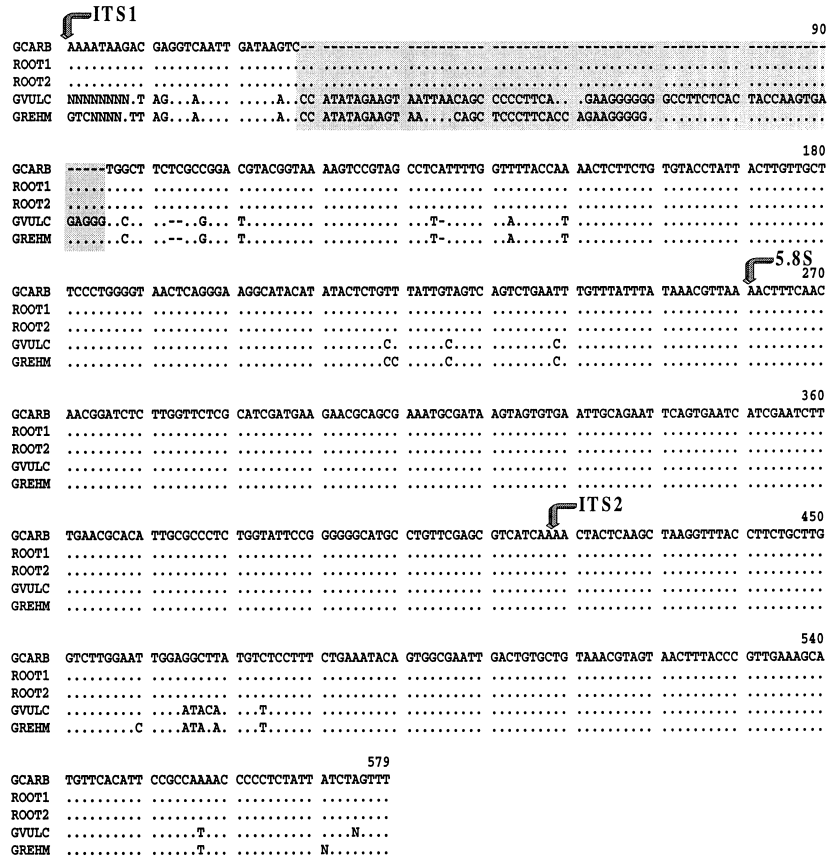

Fig. 2 The aligned sequence matrix of the nuclear ribosomal ITS1, ITS2 and the 5.8S rDNA sequences. The sequences included are a consensus sequence of *Geopyxis carbonaria* from ascospore isolates (GCARB = 1741.1), two root isolates from Norway spruce (ROOT1 = 1941.M and ROOT2 = 1942.M), *Geopyxis* cf. *vulcanalis* from apothecia (GVULC = 2156.H), and *Geopyxis rehmii* from apothecia (GREHM = 2216.H). The shaded area marks the large 'indel' in the ITS1. EMBL/GenBank/DDBJ accession numbers of the sequences are listed in Table 2.

mycelial mats increased rapidly, and the entire Petri dish was covered after 3–4 days. Production of conidia and chlamydospores was observed after 3–5 weeks. The conidial state was referable to the form genus *Dicyma* Boulanger (Boulanger 1897), and was characterized by branched, hyaline, smooth to incrustrated conidiophores carrying 10–20 minutely warted, ochraceous, spherical, oval or pyriform conidia (5.5–7.5 × 6–8 μm). The chlamydospores were typically clavate, smooth, 5–12 μm wide, produced on tips of hyphal bundles adpressed to or submerged in the growth medium. Most hyphae were smooth, hyaline and thin (1–4 μm broad), commonly interwoven by thicker (2.5–6 μm broad), pale-brown hyphae with a blister-like ornamentation (cf. Danielson 1982). Two of the seven culture isolates, one from each windfall sample, were selected for sequencing, and the ITS sequences were found to be 100% identical to the consensus ITS sequence of *G. carbonaria* (Fig. 2).

Discussion

Adaptations that allow postfire ascomycetes to cope with their ephemeral habitat in arid and semiarid areas are suggested to include heat-stimulated ascospores and mycostasis (Warcup & Baker 1963; Zak & Wicklow 1978; Wicklow & Zak 1979; Wicklow 1988). In boreal forests the fire frequency is relatively low (Zackrisson 1977), suggesting that in these habitats some additional mechanisms may be involved for the survival of postfire fungi in periods between fires. *Geopyxis carbonaria* is a rapid colonizer which dominates the early postfire fungi on boreal fire sites. It possesses thin-walled, hyaline ascospores that rapidly germinate and grow *in vitro* without any external stimuli. Ascospore dormancy in the moist and microbial active boreal forest soils is therefore unlikely to provide a successful survival strategy for this taxon. The patchy occurrence of ascocarps observed, i.e. the absence in the clear-felled stands and the lightly to moderately burnt forested stands, and abundance in the heavily burnt forested stands, suggested that a biotrophic association between fungus and tree may have existed in the prefire community. Spruce mycorrhiza detached from the host have been reported to stay viable for as much as 8 months in soil (Ferrier & Alexander 1985). The spruce trees of the postfire habitat were still alive for several months after the fire (T. Vrålstad, personal observation), and fresh, viable mycorrhiza-like roots from below the depth of detrimental heat penetration (Fig. 1C) could be isolated from the windfalls of Norway spruce as late as 15 months after the fire.

The observation that the ITS sequences of *G. carbonaria* were almost invariant, and that each *Geopyxis* species had a distinct ITS genotype, was concordant with studies of other discomycetes (Egger 1996; Norman & Egger 1996; Holst-Jensen *et al*. 1997). As the ITS genotype of the two root isolates was identical to that of *G. carbonaria*, we must conclude that the root isolates are indeed conspecific with *G. carbonaria*.

The congruent culture morphology and the identical ITS genotypes of the root isolates and *G. carbonaria* provide strong evidence for the hypothesis that *G. carbonaria* has a biotrophic association with Norway spruce in nature. This association, at least partly taking place at root depths below detrimental heat penetration of a forest fire, may explain why and how the fungus escapes physical damage and can survive a fire. The combined biotrophic and saprotrophic ability of *G. carbonaria* (Egger 1986) suggests a facultative nature of the biotrophic association, features which may not only be crucial for the ability to colonize deeper roots, but also for the ability to rapidly produce ascocarps after a fire. The extensive production of ascocarps which succeeds a forest fire may be triggered by a changing biotic and/or abiotic environment, e.g. the fire injury of associated spruce trees commonly leading to tree death (Zackrisson 1977), disruption of mycorrhizal associations, reduced competition in the upper rhizosphere, and altered physicochemical conditions. The release of stimulatory water-soluble compounds from charred wood, which have been found to induce germination of fire-adapted seeds (Keeley & Pizzorno 1986), may also play an important role. The fruiting pattern of *G. carbonaria* observed in the forested and deforested stands at the fire site of Maridalen indicates that both the presence of a suitable host (i.e. Norway spruce) and fatal fire injury to the host constituted crucial factors for the extensive fruiting of *G. carbonaria* which was observed. Similar postfire mass production of ascocarps of *G. carbonaria* is frequently reported (Moser 1949; Petersen 1970; Turnau 1984), and successively leads to extensive discharge of ascospores. The widespread dispersal of fungal propagules so characteristic of *G. carbonaria* may be explained as a specialized adaptation to an ephemeral postfire habitat. However, rather than a pyrophile 'mating-party' scenario, a plausible explanation may be a successful fungal escape from a dying host where the fungus no longer can maintain its biotrophic association. Ecological stress and disturbance altering the environmental and nutritional patterns are known to initiate modifications of physiological and morphological features among fungi, facilitating survival (Rayner 1994; Dix & Webster 1995). It is reasonable to believe that some 'postfire' fungi have evolved adaptations that allow them to escape from a postfire habitat, rather than to colonize it. The ability to produce conidia and chlamydospores, such as observed in *G. carbonaria*, may complement the survival and dispersal mechanisms of the species.

After a boreal forest fire, ascocarps of *G. carbonaria* disperse their ascospores to the surrounding healthy forests, making the re-establishment of a biotrophic association possible. However, by possessing features such as saprotrophic ability, rapidly germinating ascospores, and adaptations to cope with the postfire conditions, e.g. tolerance to high pH levels and low water-holding capacity of the substrate, the fungus may provide an *in situ* inoculum for the spruce seedlings which revegetate the fire sites. Surviving mycorrhizas below depths of detrimental heat penetration may also represent a possible source for biotrophic infection of germinating seedlings in the postfire community, a strategy proposed by Mikola *et al*. (1964) for mycorrhizal recolonization after heavy slash burning.

Ascomycetes as potential mycorrhizal symbionts with autotrophs in boreal forest systems are poorly investigated. Most mycorrhizal studies have been strongly biased towards the sporocarp-producing basidiomycetes, possibly overemphasizing the role of basidiomycetes as underground mycorrhizal inhabitants. Recent molecular ecological studies carried out in boreal pine and spruce forest ecosystems using ITS–RFLP patterns to compare above- and below-ground mycorrhizal species composition (Gardes & Bruns 1996; Dahlberg *et al*. 1997), revealed a considerable lack of correspondence between the above-ground community of fruit-body-producing mycorrhizal basidiomycetes and the below-ground community of mycorrhizal colonists. These studies showed that several below-ground mycorrhizal species never, or rarely, produced basidiocarps during the period of study. Mycorrhizal ascomycetes which never, or rarely, produce a sexual stage include the widespread hyphomycete *Cenococcum geophilum* (Dahlberg *et al*. 1997) which has affinity to the Loculoascomycetes based on molecular evidence (LoBuglio *et al*. 1996), the E-strain mycorrhizal species (Laiho & Mikola 1964; Laiho 1965; Mikola 1965; Danielson 1982) belonging to the discomycete genus *Wilcoxina* (Pyronemataceae, Pezizales) (Yang & Wilcox 1984; Yang & Korf 1985; Egger & Fortin 1990; Egger *et al*. 1991), which rarely produce ascocarps after local forest disturbances, and finally the ectomycorrhizal postfire discomycete *Sphaerosporella brunnea* (Pyronemataceae, Pezizales) (Danielson 1984) known as an occasional mass-producer of ascocarps on heavily burnt bonfire sites.

Egger & Paden (1986a) found that *G. carbonaria* caused weak, insignificant symptoms of pathogenic infection on seeds and germinants of lodgepole pine *in vitro*, features which were also observed for the ectomycorrhizal postfire discomycete *Sphaerosporella brunnea*. In pure culture synthesis experiments *G. carbonaria* did not form a typical ectomycorrhiza with roots of lodgepole pine, but formed

a discontinuous and thick mantel which gave an extensive inter- and intracellular infection of the epidermal and cortical root cells, causing chlorotic reactions on the seedlings (Egger & Paden 1986b). They suggested that *G. carbonaria* was moderately pathogenic, but supposed that the formation of a rudimentary Hartig net indicated that the fungus may be capable of forming mutualistic mycorrhizal symbiosis under some conditions (Egger & Paden 1986b). Under artificial conditions, ectomycorrhizal fungi may sometimes turn pathogenic, penetrating the cell walls and causing chlorotic reactions of the host tissue (Peterson & Farquhar 1994), indicating that there is a fine balance between being a mutualistic mycorrhizal symbiont and an intracellular pathogen. The inter- and intracellular infections by *G. carbonaria* of epidermal and cortical root cells of lodgepole pine *in vitro* observed by Egger & Paden (1986b), may be related to the ectendomycorrhizal infection of pine observed in *Wilcoxina* species. Mikola (1965) found that ectendotrophic mycorrhizas, also termed pseudomycorrhizas (Levisohn 1954, 1963), was the dominant mycorrhizal type in Finnish nurseries of Scottish pine, while mycorrhizas of Norway spruce did not possess ectendotrophic infections. At the same time, Laiho (1965), applying pure culture synthesis experiments, found that six species of pine consistently became ectendomycorrhizal, i.e. with both inter- and intracellular penetration, in association with the E-strain fungi (later recognized as *Wilcoxina* spp.). Contrastingly, spruce, fir, hemlock and Douglas fir became universally ectomycorrhizal, i.e. with intercellular penetration only, with the same mycobionts, suggesting that the nature of symbiosis and mycorrhizal morphology is largely determined by the phytobiont (Peterson & Farquhar 1994). Thus, the biotrophic relationship between *G. carbonaria* and Norway spruce may well be 'pure' ectomycorrhizal in nature, while associations with pines may be ectendomycorrhizal. Our field observations suggest that the association between Norway spruce and *G. carbonaria* is very common, while no such strong association with Scottish pine could be observed. We hypothesize that *G. carbonaria* may be an ectomycorrhizal associate with Norway spruce, a hypothesis which is not in conflict with prior observations concerning the fungus' behaviour with lodgepole pine *in vitro*. Pure culture synthesis between Norway spruce and *G. carbonaria* along with direct DNA extraction of mycorrhizal root tips (cf. Gardes & Bruns 1996) within the brown smooth group of mycorrhizal morphotypes will further confirm or reject this hypothesis.

The suggestion that an (ecto)mycorrhizal association with spruce is dominating in the life cycle of *G. carbonaria* implies that it may be a widely distributed below-ground species in boreal spruce forests, judging from its ubiquitous occurrence on boreal spruce fire sites. Future studies using molecular identification tools may provide new information about the life history and distribution of *G. carbonaria* along with other mycorrhizal ascomycetes thought to colonize the below-ground rhizosphere of boreal forest ecosystems.

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As part of the Ascomycete Research group of Oslo, Norway (ARON; http://biologi.uio.no/ascomycete/) the authors current research interests are ecology, systematics and evolution of the ascomycetes, with particular focus on fungus–plant interactions, and the discomycete orders Pezizales and Helotiales.
