Chapter 3
Spore Dispersal in Ectomycorrhizal Fungi at Fine and Regional Scales

Thomas R. Horton

3.1 Introduction

Like fungal pathogens, the distribution of EcM fungi is directly influenced by patterns of host specificity and the distribution of their host plants. Readers interested in host specificity are referred to Molina et al. (1992) and Molina and Horton (2015). In this chapter, I review how the life cycles of ectomycorrhizal (EcM) fungi impact their fine and regional scale dispersal (short- and medium-distance dispersal, respectively). I also consider life history traits that impact establishment following dispersal. The majority of case studies highlighted here are from coniferous ecosystems. My hope is that the material included has broad applicability with respect to the biogeography of EcM fungi, and while the species may change, the families and genera of the fungi considered are represented in EcM tree systems across the globe. I present nuances unique to wind and animal vectors with respect to spore dispersal and establishment. Finally, I close by reviewing three life history traits that help fungi in Basidiomycota overcome problems inherent to establishment after dispersal to uncolonized areas: secondary homothallism, dispersal via mycophagy, and the production of resistant propagules.

It is important to keep in mind that studying EcM fungi under controlled conditions continues to be difficult, because most EcM fungi are difficult to isolate and grow in the absence of a host plant. Most species are not easy to grow even with a host plant, and only a handful have been observed to fruit under laboratory, growth chamber, or greenhouse conditions (Nara 2008). Furthermore, germination cues of spores of most species are still largely unknown making it difficult to assess spore viability, grow single spore isolates, or perform mating studies. Knowledge of the life history traits of EcM fungi comes from genera that grow in culture including

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Amanita, *Boletus*, *Cenococcum*, *Hebeloma*, *Laccaria*, *Pisolithus*, *Rhizopogon*, *Suillus*, *Tuber*, among others (see Cairney and Chambers 1999). Absent from this list are genera that are often important in EcM plant communities such as *Amphinema*, *Cortinarius*, *Inocybe*, *Lactarius*, *Piloderma*, *Ramaria*, *Russula*, and *Tricholoma*. The inferences about the ecology of most EcM fungi are necessarily based on lessons from the first set of taxa and from field studies.

Relying on field studies is limited by our ability to detect the presence of a species in an area, let alone its relative abundance. Firstly, although the fungi may be active on roots, they may not fruit when investigators are on site in any given year. Secondly, mycorrhiza and especially active mycelia are difficult to observe in belowground samples. Fortunately, there have been great advances with the development of molecular tools that allow us to detect EcM fungi from vegetative structures such as EcM root tips, hyphae, and propagules in soil (Gardes and Bruns 1993; Horton and Bruns 2001; Peay et al. 2008), and much of the work reviewed below was derived from such approaches. However, even if below ground in its vegetative state, the chance of a soil sample containing hyphae, root tips, or propagules from a particular EcM fungal species is very low (Horton and Bruns 2001; Taylor 2002). Although some may see these issues as a source of frustration, I see them as a fascinating opportunity to tinker with methods to work with EcM fungi and develop new strategies to target outstanding questions.

There are other inherent difficulties when researching the role of spore dispersal in the establishment of a new individual. To emphasize this point, consider the following: there are good data for the release of a basidiospore via ballistosporic discharge and Buller's drop (Buller 1924; Pringle et al. 2005) but little data on the fate of a spore following its release. Spores can be trapped under field conditions following dispersal, but how far a spore has traveled, and especially which sporocarp released it, is very difficult to determine unless captured in close proximity to the sporocarp (Galante et al. 2011; Li 2005). Further, there are data on how many spores some species produce (Buller 1924), but there are only limited data on how spores actually become established as new individuals, or even how many become established at all. Indeed, experiments to isolate environmental conditions impacting spore release and germination have only been conducted on a handful of EcM fungal species (Halbwachs and Bässler 2015). As a result, conditions that affect dispersal and establishment are still largely speculative for most species.

3.2 Spore Liberation

EcM fungi that are hypogeous (fruit below ground) or produce puffballs have lost the ability to forcibly discharge their spores and instead release them passively (Table 3.1). However, most EcM fungi have two mechanisms to forcibly eject spores: ballistosporic discharge in Basidiomycota and the bursting of the asci in operculate Ascomycota. Many pathogenic fungi fruit on plant parts of their host positioned above the ground, contributing to increased dispersal distances and the

Table 3.1 Examples of genera of ectomycorrhizal fungi grouped by their fruiting habit

Fruiting habit	Release of spores	Primary dispersal mechanisms	Basidiomycota	Ascomycota	Zygomycota *s.lat.*
Epigeous	Forcible[a]	Wind	*Amanita*, *Boletus*, *Cantharellus*, *Cortinarius*, *Inocybe*, *Lactarius*, *Leccinum*, *Paxillus* *Russula*, ***Suillus***[b], *Tricholoma*, *Tylopilus*	*Geopyxis*, *Peziza*, ***Wilcoxina***[c]	
Hypogeous	Passive	Mammal mycophagy	***Rhizopogon***(*Suillus*)[d], *Truncocolumella*, *Alpova*, *Martelliela (Russula)*, *Gymnomyces (Russula)*, *Hydnagium (Laccaria)*, *Thaxterogaster (Cortinarius)*, *Hymenogaster (Cortinarius)*, *Amanita*, *Archangeliella (Lactarius)*, *Hysterangium (Gomphales)*, *Gautieria (Ramaria)*	*Genea*, ***Tuber***, *Balsamia*, *Terfezia*, *Elaphomyces*, *Choiromyces*	*Endogone*
Puffball	Passive	Wind	*Scleroderma*, *Pisolithus*		
Resupinate[e]/ Thelephoroid	Forcible	Wind	*Thelephora*, *Tomentella*, *Tylospora*		
Secotioid[f]	Passive	Wind/animal?	*Gastroboletus*, *Gastrosuillus*		

(continued)

Table 3.1 (continued)

Fruiting habit	Release of spores	Primary dispersal mechanisms	Basidiomycota	Ascomycota	Zygomycota *s.lat.*
Sclerotia[g]	Passive	Belowground	*Austropaxillus*, *Boletus*, *Cortinarius*, *Gyrodon*, *Hebeloma*, *Leccinum*, *Paxillus*, *Pisolithus*, *Scleroderma*	*Acephala*, ***Cenococcum***, *Phialocephala*	
Asexual spores	Passive	Belowground		***Wilcoxina***	*Glomus*, *Gigaspora*, *Sclerocystis*

Epigeous = above ground; hypogeous = below ground; puffball = spores enclosed in aboveground or erumpent sporocarp; resupinate = spores produced in a flat layer adhering to a surface such as a stick or log, but can also be loosely incorporated in the litter, may be epigeous

[a] Forcible discharge refers to ballistospores that are forcibly ejected from basidia (Basidiomycota) or ascospores that are forcibly ejected from asci (Ascomycetes); Passive release refers to spores that remain associated with the sporocarp but may be dispersed through mechanical means such as wind, rain, or animal traffic

[b] Bold type indicates there are species in the genus known to produce resistant propagules

[c] Wilcoxina produces sexual ascospores and asexual chlamydospores. Chlamydospores can build up in soils as a resistant propagule bank

[d] Names in parentheses are epigeous taxa thought to be most closely related to the hypogeous forms

[e] Resupinate sporocarps lie flat on the substrate without a cap or stem

[f] Like hypogeous sporocarps, secotioid sporocarps release spores passively within an enclosed hymenium. In contrast to hypogeous forms, this form includes remnant tissue derived from the stem and cap with varying levels of modification. Although they fruit above ground, they do not forcibly eject spores

[g] Dormant fungal tissue that resists decay and can survive deleterious conditions. See Smith et al. for a thorough treatment on fungi that produce sclerotia

chance to encounter a suitable host. In contrast, most EcM fungi fruit close to the ground, and even in those Basidiomycota with relatively long stems, the top of the cap is still positioned only about 15–20 cm above the ground. EcM fungi in Ascomycota that fruit above ground (epigeous habit) typically do not produce stems, and while they release spores into the air, they also do so close to the ground as exemplified by the small cup-shaped sporocarps produced by *Wilcoxina mikolae* (Fig. 3.1). Fungi that fruit close to the ground disperse spores at a fine scale, a fruiting habit that serves to maintain close proximity to suitable hosts and substrates.

Basidiospores produced by epigeous Basidiomycota are asymmetrical, a morphology related to ballistosporic discharge and the role of Buller's drop (Buller 1924; Pringle et al. 2005). The ballistosporic discharge mechanism in Basidiomycota is impressive in terms of the forces generated to release spores from the hymenium (Money 1998). However, the energy released quickly dissipates and likely does not greatly affect dispersal beyond the hymenium within the cap of the mushroom (Fig. 3.2; see also Stolze-Rybczynski et al. 2009). Those interested in spore liberation and aerial dispersal are directed to Buller (1922), Ingold (1971), Stolze-Rybczynski et al. (2009), and a recent review by Halbwachs and Bässler (2015) on Basidiomycota.

The caps of Basidiomycota are positioned such that spores fall down from the hymenium layer via gravity and most spores do not impact other gills as they fall. Some fungi such as *Amanita* spp. will reposition the caps through gravitropism to allow the spores to fall to the ground should the mushroom land on its side. Once the spores fall away from the boundary layer of the mushroom, they will be carried in wind currents. Many spores will still fall to the ground if wind currents are minimal (Nazaroff 2014) or when the caps are positioned very close to the ground or when spore bearing tissue is incorporated in the litter (e.g., in resupinate spp. such as *Tomentella* spp.). Many spores released from caps positioned 15–20 cm above the ground can still be captured close to the originating sporocarp with sticky

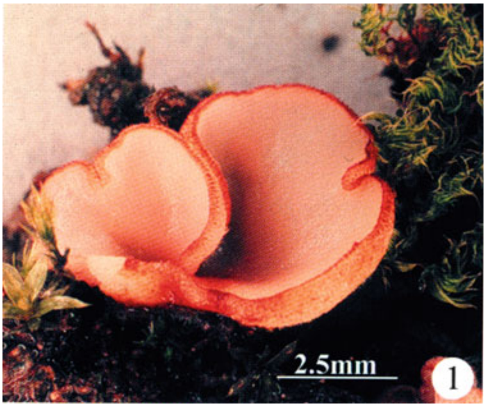

Fig. 3.1 A sporocarp of the diminutive *Wilcoxina mikolae* (note the scale bar). Modified from Trevor et al. (2001)

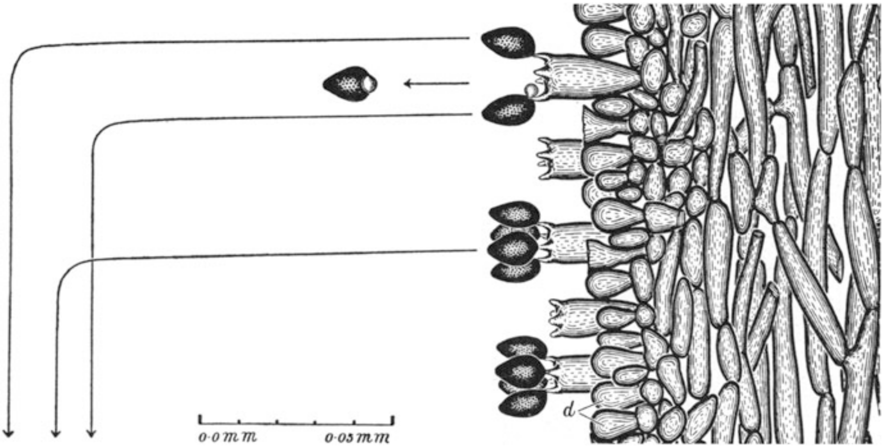

Fig. 3.2 A vertical section taken transversely to the long axis of a *Paneolus campanulatus* gill showing the probable trajectories of spores discharged from the hymenium. Three trajectories of spores, discharged in still air, are indicated by the *arrows*. Magnification, ×465. Note that only one side of a gill face is shown and the spores are not traveling far enough to impact the opposing gill face before gravity takes over. Modified from Fig. 97 in Buller (1922)

slides placed on the ground near the mushroom (Galante et al. 2011; Horton et al. 2013).

EcM fungal fruit bodies produced by Ascomycota primarily in *Helotiales* and *Pezizales* are also produced very close to the soil surface (Fig. 3.1). These spores are shot upward a few centimeters from the cup-shaped ascocarps. I am not aware of any reports documenting how far ascospores travel after being released from epigeous EcM Ascomycota such as *Peziza* or *Wilcoxina*. Interestingly, it appears that many EcM ascomycetes produce asexual propagules below ground (*Cenococcum*, *Wilcoxina*, etc.) or sexual spores in hypogeous sporocarps (*Tuber*, *Elaphomyces*, *Genea*, etc.).

3.3 Into the Air

Spores of EcM fungi are microscopic (measured in μm) and visible only in mass as a spore print from a basidiocarp or a cloud of spores from an ascocarp. While spores of many species are dispersed through the air by wind, it is very difficult to determine the distances the spores travel. Several years after Mount St. Helens erupted, Allen (1987) captured an average of one EcM spore (*Thelephoraceae* spp.) per 24 trap hours in wind traps positioned at various locations within the blast zone. Li (2005) observed less than 2% of spores released from *Amanita* sporocarps dispersed beyond a radius of 5.2 m from the source. We used microscope slides covered with a mixture of paraffin and petroleum jelly placed at various heights above the ground along transects out hundreds of meters into the open dunes from a forest edge only to capture so much debris that it was impossible to know if spores were also captured (Galante and Horton; unpublished data).

Galante et al. (2011) recovered spores that fell to the ground along 60 cm radii emanating out from sporocarps of six EcM fungus species chosen in part to reflect a diversity of sporocarp stature and spore ornamentation: *Inocybe lacera*, short stature and ridged spores; *Laccaria laccata*, short stature and spiny spores; *Lactarius rufus*, medium stature and spores with reticulate ornamentation; *Suillus brevipes*, medium stature and smooth spores; *Suillus tomentosus*, medium stature and smooth spores; and *Thelephora terrestris*, medium stature and knobby spores. Ninety-five percent of the spores observed along the horizontal transects for all species fell to the ground in the first 60 cm, with the data following a negative exponential decay curve. Dam (2013) reanalyzed the data from Galante et al. (2011) and found a variety of other models fit the data as well, and all predicted a large number of spores would be found on the ground close to the source caps. Dam (2013) suggested that basidiospores are of a size that they are influenced by the viscous drag of air and that most will be transported until they return to earth by rain (Gregory 1945). However, Nazaroff (2014) reviewed the deposition on surfaces of indoor bioaerosols 3–10 μm in diameter, the size range of fungal spores. He concluded that deposition attributable to gravitational settling onto surfaces is an important fate for such particles, even when fans generating wind up to 20 cm s^{-1} (about 0.7 km h^{-1}) were in the room. While it is likely that outdoor particle dynamics will follow the dynamics seen indoors to some extent, spores will also become entrained in faster air currents and settle away from the source sporocarp.

How many spores are released from a sporocarp, and then how many of those are carried away from the cap versus land near the cap remains speculative. Buller (1909) estimated a single sporocarp might release 1×10^9 spores, and using this figure, Galante et al. (2011) gave a conservative estimate that 1×10^7 spores could disperse in a vertical direction above the cap even if many spores fell to the ground near the source cap. In a follow-up study, vertical spore dispersal was investigated by placing sticky slides at increasing heights up to 65 cm above the caps of *Suillus luteus*. Like horizontal deposition around a sporocarp, vertical dispersal of spores fit a negative exponential decay, with 95% of the observed spores on slides within 60 cm above the caps (Sørensen et al. unpublished data).

One way to increase spore dispersal distance is to increase the height from which the spores are released. Galante et al. (2011) found that stem length was one of two characters that impacted local dispersal distance (the other being spore size). This increases the number of spores that can enter the air stream. The spores themselves have various ornamentations that may influence dispersal (Halbwachs and Bässler 2015), but the role of spore ornamentation on dispersal has not been fully explored yet.

Quantifying the airborne spores from EcM fungi is extremely difficult. Saprotrophic and pathogenic fungi can be “trapped” by placing petri dishes with homokaryotic cultures on various surfaces; the formation of a dikaryon indicates a spore of a compatible mating type landed on the homokaryotic mycelium. But EcM

Basidiomycota do not grow fast, and if homokaryotic cultures of EcM species that can be maintained in culture (e.g., *Laccaria*; Wong et al. 1989 and *Hebeloma*; Debaud et al. 1988) are placed into a field setting, they will be quickly overrun by fast-growing Ascomycota.

Using uncolonized seedlings as bait and qPCR of fungal barcodes from rainwater, Peay et al. (2012) provided important data for dispersal from 0.5 m to 5.4 km from a forest edge. They showed that there was a dramatic decline in spores captured between the first 10 and 100 m from the source of spores for the three most abundant EcM fungi: *Suillus pungens*, *Thelephora terrestris*, and *Tomentella sublilacina*. The fact that the spores were collected in rainwater is interesting as this lends support to the idea that airborne spores can be washed out by precipitation (Gregory 1945; Ingold 1971) and can thus be carried over considerable distances in wind currents until a rain event. The observation of *Tomentella sublilacina* spores in the study is also interesting as this fungus has a resupinate habit. This leaves open the question of how the spores became airborne. Perhaps spores were carried by invertebrates (Lilleskov and Bruns 2005).

3.4 Short-Distance Wind Dispersal: Home Is Where the Roots Are

A new genet establishing following short-distance dispersal benefits from being in the area where conditions supported the growth and fruiting of the species (the thallus that yielded the mushroom). The location is already occupied by compatible host trees, and the edaphic conditions are suitable for that species of EcM fungus. However, the genet is also attempting to establish in a location with other EcM fungi, making it difficult to find uncolonized roots, particularly in the face of competition with previously established thalli.

While the maximum size of EcM individuals (strain, clone, genet) can be on the order of meters to tens of meters, the mean size is typically less than 3 m (Douhan et al. 2011; Lilleskov et al. 2004; Chap. 2). An EcM fungus genet does not completely fill the soil volume occupied—anyone who has sorted and identified EcM fungi from root tips knows that multiple species will occur on adjacent root clusters at the scale of mm. It is likely that a genet is distributed patchily throughout the volume of soil with clusters of roots connected by sparse networks of hyphae (Agerer 2001) and that most of the soil is not occupied by the strain even within the boundaries of the thallus. A new genet may very well find uncolonized root tips to establish, even in the same general location as that occupied by the mother thallus. Shiros (or castles; fairy rings produced by *Tricholoma matsutake* and related species) provide good evidence of single strains occupying large areas, at least in occupied rings (Chap. 15). However, some shiros are composed of multiple genets (Lian et al. 2006), possibly the result of the establishment of new genets following spore release from the original or mother thallus. The local population structure of other EcM fungi (those that do not necessarily produce classic fairy rings) are also typically a patchwork of individuals (Beiler et al. 2010; Dunham et al. 2003; Kretzer et al. 2004, 2005) with multiple genets and species that compete for root tips and other resources at a fine scale.

The ability to differentiate between parental, sibling, and other genotypes in EcM fungi remains difficult. One reason being, as pointed out by Fries (1978), it is notoriously difficult to induce spore germination of most EcM fungi, a necessary step when sorting out the sexual compatibility system of a species. Somatic compatibility tests are also difficult because many of the fungi do not grow well in culture even as dikaryons (Bonello et al. 1998). Further, highly variable microsatellite markers are not abundant in EcM fungi and microsatellite markers have been developed in only a handful of EcM taxa (Douhan et al. 2011). As Fries suggested over 30 years ago, understanding the genetic system of these fungi will lead to a greater understanding of their biology (Fries 1987), and we are still working toward that goal for many EcM species.

3.5 Medium-Distance Wind Dispersal: How Far Is Too Far?

Spores that disperse via wind face a major limitation to establishing a new individual if they are transported to uncolonized areas. The vast majority of EcM fungi cannot be maintained in culture without living host roots. This is good evidence that a propagule (spore, sclerotium, chlamydospore) from most EcM fungi may begin to form a new thallus, but the individual will not survive without a host. EcM fungi in Basidiomycota face an additional critical impediment to establishing a new individual from a single spore. Although some Basidiomycota can form functioning mycorrhizal roots as monokaryons (Gardes et al. 1990; Kropp et al. 1987; Kropp and Fortin 1988), the majority of species are thought to form vigorous mycorrhizae only as dikaryons. This means that a single spore germinant is not likely to survive long if the species is not already established in the area even if compatible hosts are present. Hyphae from a Basidiomycota spore must encounter haploid hypha from a compatible strain in order to form a dikaryon (functionally equivalent to a diploid but the two compatible nuclei remain independent). A major limitation to successful establishment following medium-distance dispersal is the low probability of encountering germinants of compatible strains. This is not a problem with local dispersal because so many spores are present from the source sporocarp and other sporocarps in the area. Even if encountering spore germinants only from the source genet, 25% of those spores will be mating-type compatible in fungi with a tetrapolar mating system and 50% of the spores will be compatible for fungi with a bipolar mating system. Three species of *Laccaria* are known to have a tetrapolar mating system (Doudrick and Anderson 1989; Kropp and Fortin 1988), while *Rhizopogon* *rubescens* has a bipolar mating system (Kawai et al. 2008). Because of the difficulties with inducing germination and identifying successful matings (dikaryons) in EcM Basidiomycota, the mating system of most EcM fungi has yet to be elucidated (Kawai et al. 2008).

The chances of encountering spores of the same species after dispersal by wind may be low irrespective of the mating system. If the new location lacks other individuals of the same species, the source of spores may be from the area that yielded the dispersed spore. But when spores are dispersed in wind, the spore rain becomes increasingly diffuse with distance (Galante et al. 2011; Peay et al. 2012). Very few spores will be encountered in the new location unless there has been time for a resistant propagule bank to develop in the soil.

Peay et al. (2012) reported less than one spore per cm^2 per day was captured 1 km from the source location. It is reasonable to assume that as the distance increases from the source of the spores, the chance of encountering spores from the same source in the new location becomes more remote. This diffuse spore rain likely played a large role in the reduction of colonization on seedlings placed at increasing distances from the source location in the study by Peay et al. (2012). It is unclear where the break point is for dispersal distance at which the probability is too low for enough spore rain to support establishment, but very few spores appear to disperse at the scale of km (Peay et al. 2012) even for the most prolific producers of spores such as species of *Suillus* (e.g., spores per mushroom and number of mushrooms fruiting in an area). However, dispersal limitation does not tell the whole story. Species of *Clavulina*, *Cortinarius*, and *Tricholoma* were relatively abundant in spore traps in Peay et al. (2012) but were not observed on mycorrhizal roots of the bait seedlings. It appears some unknown factor or factors limit most EcM fungi from establishing new genets after dispersal to new areas, perhaps related to spore behavior, interactions with more competitive species under the conditions at the site, or simply the physiological interaction between these species and their hosts as seedlings (Last et al. 1987).

3.6 Ascomycota

EM fungi in Pezizales (Ascomycota) occur in the vegetative state as haploids, and a single spore can lead to a functioning individual following dispersal. Indeed, EcM roots formed by species of Ascomycota are colonized by haploid mycelia. Ultimately, the interaction of two compatible mating types is needed to complete the life cycle with plasmogamy and karyogamy occurring in the ascocarp followed by meiosis and a post-meiotic mitosis to form eight haploid spores in each ascus. In contrast to Basidiomycota, in Ascomycota the two sexually compatible haploid mycelia remain independent ecological entities during the majority of the life cycle, reducing the negative effect of a diffuse spore rain. This may in part explain why

EcM Ascomycota are commonly encountered on plants in disturbed habitats and primary successional settings where mycelial networks are lacking and EcM fungi must establish through spore inoculum.

3.7 Secondary Homothallism

About 1% of spores in some *Suillus* spp. are binucleate (Bonello et al. 1998; Horton 2006), which is the same percentage of spores that germinate and are dikaryotic based on the presence of clamps in the germinants (Bonello et al. 1998). The production of dikaryotic spores is achieved through secondary homothallism, essentially a mechanism for self-fertilization. In Basidiomycota, a post-meiotic mitosis occurs during spore production resulting in eight nuclei, a feature of the life cycle not shown in most textbooks (Malik and Vilgalys 1999). The mitotic division may occur in the spore, sterigma, or basidium. If mitosis occurs in the basidium, eight nuclei can migrate into the developing spores with the possibility that two nuclei of compatible mating types may be packed together, resulting in a dikaryotic spore. Jain (1976) put forth the reproductive assurance hypothesis for plants suggesting that there is a selective advantage to selfing if a single propagule can establish a new viable population after dispersal to an uncolonized location even though selfing may lead to inbreeding depression. Evidence for selfing in Basidiomycota is scant, but has been shown in *Suillus*, *Laccaria*, and *Hydnagium* (Bonello et al. 1998; Jacobson and Miller 1994; Mueller et al. 1993; Treu and Miller 1993). Although many species produce binucleate spores, most nuclei in such spores are derived from a mitotic division following migration of a single nucleus into the spore, which can only form a haploid mycelium. Interestingly, a low percentage of binucleate spores are produced even in fungi that produce uninucleate spores, raising the possibility that dikaryotic spores may result from nucleus packaging errors during spore development (see Horton 2006 for more on EcM fungi that produce binucleate spores). It is unclear whether the production of dikaryotic spores is as beneficial to EcM fungi as selfing is to pioneer plant species, but this may contribute to the success of *Suillus* and *Laccaria* spp. in early successional settings.

3.8 Mycophagy

Another way EcM fungi increase chances for establishment following spore dispersal is through mycophagy. Spores of many EcM fungi are dispersed by animals that consume the sporocarps and deposit the spores in their feces. There are many records of mammals eating epigeous and hypogeous fungi or the spores being found in stomach contents or fecal samples (Cazares and Trappe 1994; Colgan and Claridge 2002; Fogel and Trappe 1978; Izzo et al. 2005; Luoma et al. 2003; Maser et al. 1978). Alsheikh and Trappe (1983) reported a bird species eating desert truffles. Ashkannejhad and Horton (2006) found spores of both epigeous and hypogeous fungi (*Suillus* and *Rhizopogon* spp., respectively) in deer fecal pellets, and, importantly, pine seedlings inoculated with slurries made from the deer pellets yielded seedlings colonized by *Suillus* and *Rhizopogon* spp. Large mammals such as deer and wild boar can disperse spores up to several kilometers. Deer and boar fecal pellets can contain millions of spores (Ashkannejhad and Horton 2006; Nuñez et al. 2013) many of which are mating-type compatible, suggesting that dispersal of spores via mycophagy by large mammals has important advantages over the diffuse spore rain dispersed via wind over similar distances.

On Isla Victoria, Argentina, conifers were not establishing outside the perimeter of plantations despite the fruiting of compatible EcM fungi introduced with the conifers in the plantations (Nuñez et al. 2009; Simberloff et al. 2002). This pattern revealed two things. Firstly, the conifers were not associating with native EcM fungi associated with *Nothofagus*, at least not to the extent that supported establishment (Hayward et al. 2015a). Secondly, wind dispersal of spores from fungi fruiting in the plantations was not leading to conifer invasion into native *Nothofagus* stands. However, European wild boar and deer were recently introduced on the island and have established growing populations. Now the introduced mammals are eating the conifer-specific EcM fungi in the plantations. Seedlings inoculated with boar or deer fecal pellets collected during the fruiting season outside the plantations yielded conifer-specific EcM fungi on the seedlings (Nuñez et al. 2013). It appears as if the introduction of the conifers and their specific EcM fungi was not enough to lead to an invasion, probably because of dispersal limitations of the fungi (Nuñez et al. 2009). However, the conifers are now spreading into the native *Nothofagus* stands, suggesting that mammalian dispersal vectors of the EcM fungi were necessary for the invasion to proceed (Nuñez et al. 2013). Although the Northern Hemisphere conifers were able to associate with a few EcM fungi associated with in the Southern Hemisphere *Nothofagus*, they still could not establish in those stands. Like the primary successional system investigated by Ashkannejhad and Horton (2006) in Oregon, USA, the fungi supporting the spread of the conifers were primarily suilloid species in the genera *Suillus* and *Rhizopogon* that were introduced with the pines to Isla Victoria and dispersed by large mammals.

Spores dispersed by mycophagist mammals must survive passage through the digestive track of the animal to remain viable as inoculum. This same feature of resistance may allow the spores to lie dormant for an undetermined amount of time in soils. Ashkannejhad and Horton (2006) found spores of *Suillus* and *Rhizopogon* remained viable as inoculant in dry fecal pellets stored at room temperature for 1 year. Bruns et al. (2008) found that spore inoculum potential of four *Rhizopogon* spp. increased with time over a 4-year period.

3.9 Resistant Spores

The production of resistant spores that can remain dormant in soils may be another important life history trait that increases the chance for establishment of EcM fungi following dispersal. Horton (2006) used DAPI stain to observe the number of nuclei in spores collected from spore prints for a large number of EcM fungus species across multiple genera. While nuclei were observed in fresh spores of all fungi, spores from the same spore prints did not show nuclei after storage for 1 year at room temperature except *Rhizopogon* and *Suillus*. These data support other evidence suggesting that the spores of most EcM fungi are relatively short-lived but that *Rhizopogon* and *Suillus* produce spores are resistant and may form dormant spore banks in soils (Baar et al. 1999; Bruns et al. 2008; Horton and Bruns 1998).

EcM fungi that generate a resistant spore bank benefit from dormancy in an analogous way that plants benefit from soil seed banks (Simpson et al. 1989). Both *Suillus* and *Rhizopogon* appear to produce resistant spores, but *Rhizopogon* spp. may be unique in their dormancy mechanisms (Nara 2008). Horton et al. (1998) and Baar et al. (1999) sampled pine seedlings establishing after a stand replacing fire. *Rhizopogon* and to a lesser extent *Suillus* were the primary EcM fungi found colonizing postfire seedlings. This was in contrast to belowground studies from the area prior to the fire showing a variety of EcM fungi in the genera *Russula*, *Lactarius*, *Amanita*, *Laccaria*, and *Boletus* on mature trees and a near absence of *Rhizopogon* and *Suillus* (Bruns et al. 2005; Gardes and Bruns 1996; Horton and Bruns 1998; Taylor and Bruns 1999). These results supported results in Taylor and Bruns (1999) who showed a shift from a complex assemblage of fungi on in situ Bishop pine roots harvested in soil cores from the mature forest to a community dominated by *Rhizopogon* and *Suillus* on Bishop pine seedlings grown in soils from the same cores in a bioassay experiment. Mycorrhizal infection was likely from mycelial networks in the mature forest while the soil bioassay method selected fungi with resistant propagules in the soils, mostly spores but also sclerotia (e.g., *Cenococcum*) or chlamydospores (e.g., *Wilcoxina*). Removal of soils from the forest disrupts the mycelial networks and kills them by severing the mycelia from the host roots (carbon source). The hyphae may be infective initially (Horton et al. 1998) but appear to lose efficacy as soils dry out. However, EcM fungi that produce resistant propagules are able to survive the death of the host and drying of the soils and can colonize seedlings in bioassay experiments (see Table 3.1). This same feature enables these EcM fungi to survive lengthy dispersal events, facilitating establishment in new locations.

3.10 Conclusions and Future Perspectives

The understanding of spore dispersal in EcM fungi remains somewhat speculative for a number of reasons. Spores are difficult to track in field settings, and spores of most EcM species do not germinate readily if at all under laboratory conditions.

The fungi cannot be easily grown in lab even with a compatible host. The fungi are difficult to observe from samples of mycelia or mycorrhizal root tips collected from the field. Further, the sporocarps of many species may not be easily detected because of their cryptic nature and sporadic and ephemeral fruiting habits. However, applications of various PCR-based methods are providing insights about the distribution of the fungi, their population structure, and knowledge about establishment in new areas.

Spores of epigeous taxa are primarily dispersed via wind. Airborne spores may be deposited in close proximity to the sporocarp or be carried over considerable distances by wind. Spores that become airborne may be deposited with gravity on surfaces or remain airborne until a rain event washes them out of the air, but more empirical evidence is needed for airborne or water-borne spore deposition of EcM fungi.

Once spores are airborne, they likely become increasingly diffuse with dispersal distance. This may impact fungi in Basidiomycota more than Ascomycota because Ascomycota are haploid in the vegetative state and only need a single spore to establish a new thallus. Spores of Basidiomycota may not be able to successfully establish in uncolonized locations, because the hyphae of a haploid germinant are believed to be short-lived (but some species can form inferior mycorrhiza as haploids). A diffuse spore rain reduces the chance for hyphae from a germinating Basidiomycota spore from encountering hyphae from a compatible spore to form a dikaryon in uncolonized locations.

While many species of EcM fungi in Basidiomycota form binucleate spores, only a few are known to produce dikaryotic spores through secondary homothallism. The fitness cost of selfing for those that undergo selfing through secondary homothallism may be outweighed by gains from establishment in new areas. Species that produce dikaryotic spores may be able to establish easier after dispersal to uncolonized areas, because the germinant is a dikaryon and can form functioning EcM associations as long as compatible hosts are available.

Animal dispersal is relatively common in EcM fungi. The animals eat the sporocarps and pass viable spores through the digestive tract. Large mammals such as deer and boar can disperse spores over hundreds and sometimes thousands of meters. The fecal material can contain millions of spores from a single species but also contain a mix of spores from multiple species. Because there are so many spores of a species in the feces, the chance of a germinant encountering mating-type compatible spores is high and so fungi dispersed via mycophagy have an excellent chance of establishment in new locations, again, as long as roots of a compatible host plant are available.

Some EcM fungi produce resistant propagules in the form of sexual and asexual spores, as well as sclerotia. These propagules can lie dormant in soils until conditions or a suitable host becomes available. How long such propagules can survive in soils is not well documented. Spores from some species of *Rhizopogon* appear to become more viable over time in the dormant state.

Finally, it is interesting that the life history of *Suillus* and *Rhizopogon* spp. includes features that appear to contribute to successful establishment in new locations, particularly resistant propagules and secondary homothallism. These same life history features may help explain why these fungi are often coinvasive with Pinaceae (Chu-Chou and Grace 1983a, b; Hayward et al. 2015a, b; Nuñez et al. 2009). The fungi were initially transported by humans to the Southern Hemisphere in nonsterile soil or litter from host stands or plantations in the Northern Hemisphere. Understanding the invasion biology of this model system may lead to insights into invasions of other mycorrhizal plants (Nuñez et al. 2008).

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