Host selection in European ectomycorrhizal Basidiomycetes affects their estimated spore volumes.

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Abstract

Many ectomycorrhizal fungi show selective associations with certain host tree taxa. Their significance has been demonstrated in the vegetative growth of the fungi, such as structuring the fungal community of the mycorrhizal roots. On the other hand, our understanding of the effects of host selection on reproductive traits such as pore production is very limited. Using two independently published descriptions of host associations and spore dimensions, the differences in spore volumes depending on the associated host taxa were examined in 10 ectomycorrhizal genera. *Suillus* was the most specific genus while *Inocybe* was a generalist. *Suillus*, *Lacterias*, *Russula*, and *Hebeloma* produced significantly ($P<0.05$) larger spores when they associated with certain host taxa or groups, including Pinaceae or broad-leaved hosts. However, such differences were not found in *Leccinum* and *Tricoloma*. The possibility that the host selection of ectomycorrhizal fungi provides an ecological advantage by producing larger volumes was discussed.

Keywords: *Amanita*, *Cortinarius*, host-mycorrhizal interaction, host specificity, *Hygroporus*

Ectomycorrhizas are a symbiotic lifestyle of the soil fungi with woody plant hosts, including Pinaceae, Fagaceae, Betulaceae, and Salicaceae (Alexander, 2006). The recent analysis reporting that more than half of the ectomycorrhizal fungi are associated with two or fewer host plant genera (Voller et al., 2023) suggests many ectomycorrhizal fungi are selective in their host plant taxa. The host selection of the ectomycorrhizal fungi plays a pivotal role in structuring the fungi's belowground community (Ishida et al., 2007; Matsuoka et al., 2020). Additionally, the host specificity has been demonstrated to contribute to the dominance of the specialised fungi in the soil fungal community (Tedersoo et al., 2024) by providing putative competitive advantages to the fungi (Kennedy et al., 2020). Moreover, the host specificity facilitates nutrient exchange between the host and the fungi (Finlay, 1989). Therefore, the importance of host selection of ectomycorrhizal fungi on their vegetative growth in the soil is well recognised whereas its influence on aboveground reproductive traits, such as spore productions, is still poorly understood, despite the previous research demonstrating that some of the reproductive traits of the ectomycorrhizal fungi were influenced by the host tree. For instance, the host tree sizes affect sporocarp production of associated ectomycorrhizal fungi (Nara et al., 2003) and symbiotic ectomycorrhizal fungi yield larger sporocarps compared to solitary saprotrophs through the possible supply of

resources from the hosts (Bässler et al., 2015). Therefore, it is likely that the other reproductive traits of the ectomycorrhizal fungi, such as spore production, are affected by the specific host taxa.

In this study, I tested the possibility that the spore volumes show any differences depending on the host taxa with which they are associated. Ten ectomycorrhizal genera, including *Amanita*, *Cortinarius*, *Hebeloma*, *Hygroporus*, *Inocybe*, *Lactarius*, *Leccinum*, *Russula*, *Suillus*, *Tricoloma*, were selected for the present study because they are proven to be ectomycorrhizal (Comandini et al., 2012), species-rich, show some host specificity and produce large sporocarps with measurable spores.

Descriptions of the associating host taxa and two-dimensional (length and width) spore size measurements were extracted from the books by Læssøe and Petersen (2019) and by Kibby (2020–2023).

To obtain a sufficient number of species for statistical analysis, the host tree taxa were classified by the family level for *Cortinarius*, *Hygrophorus*, *Lactarius*, *Leccinum*, *Russula* and *Tricoloma*. For less selective fungi, such as *Amanita*, *Hebeloma*, and *Inocybe*, the hosts were grouped into broad-leaved only or both broad-leaved and coniferous species. For highly specific *Suillus*, the hosts were divided into genus level, such as *Larix* and *Pinus*. The comparison was made between two major host tree taxa or

groups for each ectomycorrhizal genus. To estimate a degree of host specificity in the genus of interest, the proportions of the species associated with a single genus of the host tree were calculated.

The midpoints of length and width measurements were employed to calculate the spore volume for each species (Bässler et al., 2015), using the formula by Calhim et al. (2018). For the volume estimations, the shape of the spores was assumed to be an ellipsoid (Bässler et al., 2015), except for some species of *Amanita* whose shapes were spherical. In that case, the volume was calculated by the formula of $V=4/3*3.14*r^3$.

Brunner-Menzel test was performed to make statistical comparisons using Jamovi software package (The Jamovi project, 2022).

Among the fungal genera examined, *Suillus* was the most specific genus and all species were associated with a single host tree genus. On the other hand, the least specific i.e., generalist, was *Inocybe* with less than 20% of a single genus associated species (Fig. 1). The spore volumes in *Suillus* were significantly ($P<0.05$) influenced by the different host genera in both descriptions by Kibby and by Læssøe &Petersen. However, those in *Inocybe* were statistically indistinguishable between the hosts by either description. *Suillus*, *Russula*, *Lacterius* and *Hebeloma* were the genera that statistically supported host effects on spore volumes were detected in both descriptions. A general trend that

increased host specificity positively associated with the robustness (supported by two descriptions) of host influence on the spore volume was observed. The interesting genera were *Leccinum* and *Tricoloma*, which showed high (>50%) host selection but no statistically significant host effects. *Leccinum* has been considered to shift their hosts recently (den Bakker et al., 2004), which may not provide sufficient time to develop the host effects on spore volumes. *Tricoloma* spp. have been shown to form mycorrhizas between oak and pine in vitro (Yamanaka et al., 2014). Thus, the observed selectivity in the field descriptions may reflect the environmental conditions such as the availability of the host trees in the locations they grow. These two instances of *Leccinum* and *Tricoloma* are revealing; suggesting that stable host-fungal associations and exclusive affinity between the host and the fungi are required to manifest the host effects on the spore volume.

In *Russula* and *Lactarius*, the spore volumes were significantly ($P<0.05$) larger with Pinaceae hosts than those with Fagaceae or Betulaceae hosts in both descriptions (Table 1). Additionally, *Hebeloma* associated with broad-leaved hosts and *Suillus* with *Larix* produced significantly larger spores than those with other hosts such as those found with both broad-leaved and coniferous hosts or *Pinus* respectively (Table 1). Moreover, *Cortinarius*, *Hygroporus*, and *Amanita* produced larger spores when they associated

with Pinaceae or broadleaved hosts although statistical significance was observed in only one of two descriptions (Table 1). The previous studies have shown that the larger spores were thought to retain more water and provide some protection against desiccation (Kauserud et al., 2011) and spores become larger in resource-limited conditions in the field (Halbwachs et al., 2017). These lines of evidence suggest the possibility that having larger spores increases the chance of spore survival under harsh and resource-constrained environments by preventing desiccation and storing larger amounts of nutrients to be viable for a long period until encountering the host roots. In line with this, producing larger spores by associating with particular host trees may provide competitive advantages to the fungi for efficient ectomycorrhizal formation when the conditions are unfavourable. Indeed, *Larix* spp., the host for highly specific *Suillus*, grows under dry, cold winter conditions in native high Alpine rages and usually performs better in poor to medium nutrient sites as an early coloniser in the lower elevation (Da Ronch et al., 2016). Thus, producing larger spores in *Suillus* associated with *Larix* spp. may reflect fungal adaptation for increasing the probability of survival under such limited conditions.

Russula and *Lacterius* were known to be “late-stage” fungi (Smith & Read, 2008), and their sporocarps appear at older stand ages in *Pinus sylvestris* forests (Bonet et al.,

2004). Whereas “late-stage” fruiting may be due to slow developments of belowground fungal biomass, the late fruiting may be caused by delayed spore germination, waiting for the time at which the host tree reaches a certain age. If this is the case, larger spores can contribute to remaining viable for a longer period by waiting for the host with suitable ages to be around.

The ecological significance of larger spores in *Hebeloma* is not yet clear while the genera showed robust statistical support for host effects on spore volumes (Table 1). The previous study showing that a specific association between *Suillus* and *Larix* facilitated nutrient exchange (Finlay, 1989) suggests the possibility that *Hebeloma* has a higher affinity to broad-laved hosts and produces larger spores by obtaining more nutrients from the host tree.

The spore volumes of *Amanita*, *Cortinarius*, and *Hygrophorus* increased in association with the hosts of broad-leaved or Pinaceae respectively (Table 1) although only one of the two descriptions, namely by Kiddy, was statistically supported. As Kiddy describes mainly British species, this may reflect more specialisations of these genera in Britain to the particular host taxa by geographical isolation from mainland Europe. The geographical isolation by growing on an island, such as Corsica, can change the relationship between ectomycorrhizal fungi and their host tree by shifting the host

ranges of the fungi (Rochet et al., 2011). Such an instance suggests the possibility that the separation of Britain from mainland Europe may also influence the host effects on spore volumes although this requires more detailed examinations.

The results in this study showed that spore volumes of some ectomycorrhizal fungi were affected by taxonomical differences of the host tree. It is interesting for future research to ask the questions about whether other reproductive traits, such as time of fruiting or fruitbody sizes may be affected by the different host taxa.

Disclosure

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Table 1 Comparisons of the estimated mean spore volumes (μm^3) of 10 ectomycorrzal fungal genera occurred between two different host taxa or groups

Fungal genus	Host tree		P-value*	Reference
Amanita	Broad-leaved	Broad-leaved & coniferous		
	568 (21)	358 (14)	0.004	K
	632 (18)	417 (9)	0.101	L&P
Cortinarius	Pinaceae	Fageceae		
	146 (87)	212 (66)	0.001	K
	168 (30)	188 (33)	0.169	L&P
Hebeloma	Broad-leaved	Broad-leaved & coniferous		
	261 (24)	196 (12)	0.036	K
	262 (8)	200 (4)	0.048	L&P
Hygrophorus	Pinaceae	Fageceae		
	152 (13)	110 (11)	0.042	K
	138 (11)	112 (10)	0.338	L&P
Inocybe	Broad-leaved	Broad-leaved & coniferous		
	204 (67)	179 (25)	0.389	K
	203 (28)	173 (20)	0.112	L&P
Lactarius	Pinaceae	Betulaceae		
	204 (13)	147 (15)	0.002	K
	231 (14)	129 (11)	0.001	L&P
Leccinum	Betulaceae	Other genera		
	235 (5)	155 (4)	0.208	K
	232 (6)	174 (5)	0.24	L&P
Russula	Pinaceae	Fageceae		

	249 (18)	187 (55)	0.001	K
	266 (14)	199 (39)	0.001	L&P
Suillus	*Larix*	*Pinus*		
	93.8 (6)	59.4 (8)	0.041	K
	103 (6)	65 (9)	0.021	L&P
Tricoloma	Pinaceae	Fageceae		
	84 (20)	86.8 (13)	0.306	K
	76.2 (15)	85.1 (7)	0.381	L&P

* P-values were calculated by Brunner-Munzer test.

The numbers of species examined were indicated in round brackets.

In reference, K and L&P indicate Kibby (2020–2023) and Læssøe & Petersen (2019)

Fig 1

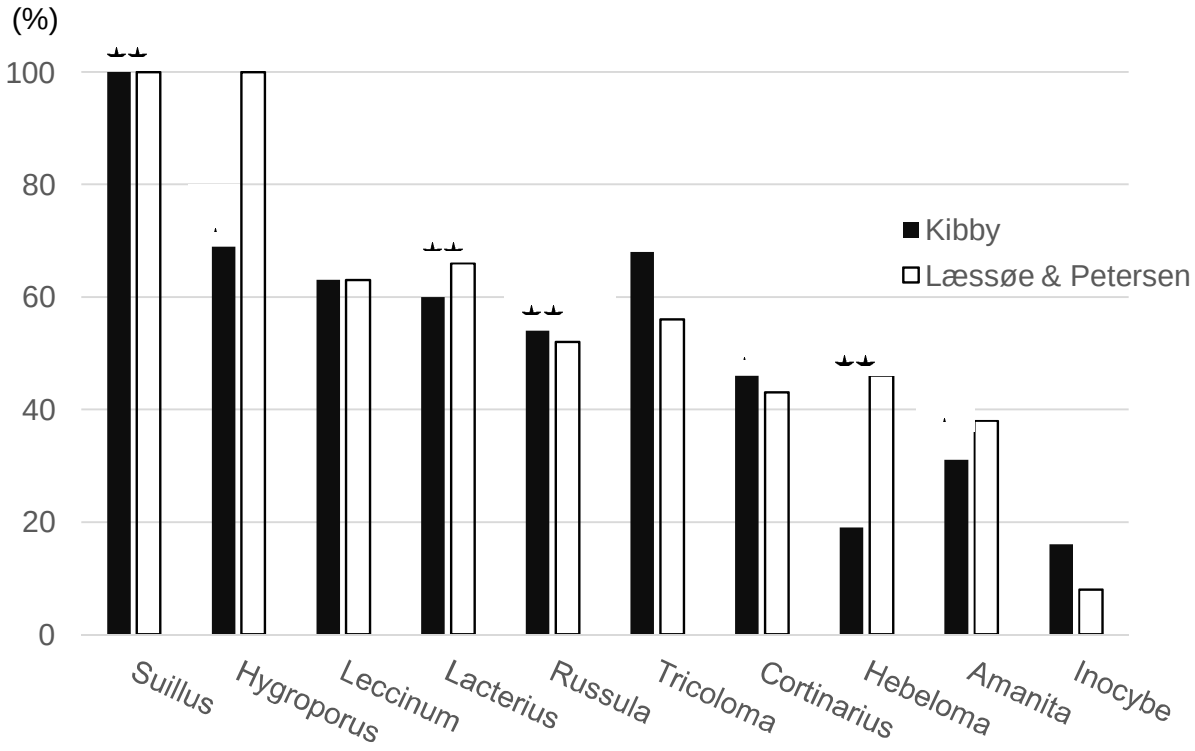

Fig. 1 Proportions of the fungal species occurring with a single genus of the host tree. Two independent descriptions of the species by Kibby (2020–2023) and Læssøe and Petersen (2019) are shown in closed and open bars respectively. The star on the bar indicates the fungal descriptions that statistically supported ($P<0.05$) spore volume differences are observed between species occurring in different host taxa.