Inherent species characteristic influence and growth performance assessment for mycelium composite applications

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Abstract

Composite materials produced using mycelial growth attract commercial and academic interest due to their economic, environmentally sustainable and green manufacturing process. However, their manufacture via slow biological growth affects the larger scale production viability of these materials, which must compete with rapidly producible synthetic materials. Hyphal characteristics vary significantly by species, which is the most influential growth performance factor in conjunction with environmental conditions and chemical nutrition. This study assessed the effect of potential growth predictors such as hyphal type, pathogenicity, taxonomic and association based classification systems on hyphal extension rate and growth density for commonly used and non-traditional species. It provides a simple, low-cost process for screening species by growth performance prior to more application-dependent mechanical evaluation. This facilitates more efficient and accurate species selection for composite manufacturing applications. Trimitic and dimitic species containing skeletal hyphae exhibited higher hyphal extension rates than species containing generative-binding or purely generative hyphae but no other parameters investigated in this study were good predictors for growth performance with significant species-specific variation present instead. However, the methodology used to test growth performance did prove effective and could be used on a case by case basis for growth screening in mycelium composite applications.

Keywords: Inherent species characteristics, species selection methodology, growth performance assessment, mycelium, composite.

Introduction

Increasing government and public environmental awareness have driven both academic and commercial interest in mycelium composites over the past decade (Jones et al. 2017). Mycelium is the vegetative growth of filamentous fungi that bonds organic matter through a network of hyphal micro-filaments to produce economical and environmentally sustainable biocomposites (Jiang et al. 2014). This natural biological growth acts as a low energy manufacturing process enabling the production of environmentally friendly alternatives to synthetic planar materials (e.g. plastic films and sheets) (Haneef et al. 2017), and larger low density (light weight) objects (e.g. synthetic foams and plastics) (Holt et al. 2012; López Nava et al. 2016; Pelletier et al. 2013; Travaglini et al. 2013).

For mycelium composites to compete with traditional synthetic materials, they must exhibit comparable material properties and be rapidly producible. Although several recent studies have investigated the material properties (Haneef et al. 2017; Holt et al. 2012; López Nava et al. 2016; Pelletier et al. 2013; Travaglini et al. 2013) and manufacturing procedures (Jiang et al. 2016a; Jiang et al. 2016b) of mycelium composites, inherent factors affecting production rate have not yet been addressed. Production rate is particularly important because manufacturing via natural biological growth is inherently slow compared to traditional manufacturing processes and limits the large-scale viability of mycelium composite production.

Fungal species used, in conjunction with environmental conditions and chemical nutrition, is the most influential growth performance parameter (Kavanagh 2005) and is hence important to the production rate. Existing mycelium composites research has exclusively utilised easily sourced basidiomycetes from the *Pleurotus* and *Ganoderma* genera (Haneef et al. 2017; Holt et al. 2012; López Nava et al. 2016; Pelletier et al. 2013; Travaglini et al. 2013). With 80,000 to 120,000 documented species (Webster & Weber 2007), of some 1.5 million (Hawksworth 2001) to 5.1 million fungal species (Blackwell 2011) estimated to be in existence, such limited and arbitrary selection is too random. However, the existence of such a large number of species makes species selection very challenging.

Basidiomycetes contain up to three distinct hyphal types, namely generative, binding (also known as ligative) and skeletal hyphae (Corner 1953). The number of different hyphal types present in a species is described using the mitic system. Monomitic species comprise of

only generative hyphae; dimitic species comprise of two hyphal types, and trimitic species comprise of all three principle hyphal types (Webster & Weber 2007). Each hyphal type exhibits different degrees of branching. Skeletal hyphae are unbranched or very sparsely branched, generative hyphae are moderately branched, and binding hyphae are highly branched (Breitenbach & Kränzlin 1986; Webster & Weber 2007). Degrees of branching and hyphal extension rates are inversely proportional in ascomycetes due to increased utilisation of substrate and inhibitory staling compounds produced (e.g. aldehydes) (Robinson & Park 1966) as hyphal density increases meaning the hyphal extension rate is insufficient to allow growth into new areas of the substrate (Prosser 1993). The hyphal growth unit (G), which is a property of the mycelium that is mathematically linked to other hyphal and colony growth parameters (Kotov & Reshetnikov 1990), also increases as branching becomes sparser (Prosser 1995). As such, hyphal types present may potentially be a growth performance predictor that could assist in species selection to increase mycelium composite production rates.

Pathogenicity, which describes an organism's ability to attack and infect a host, could also potentially be used to assist species selection and increase production rates. Many pathogens use specialised toxins, enzymatic degradation, subversions of cellular processes, mechanical forces, or a combination of these, to rapidly invade and colonise host material (Kavanagh 2005; Sexton & Howlett 2006). While animal pathogens typically only cause serious fungal infections in immunocompromised hosts, plant pathogens have mechanisms allowing invasion of even healthy hosts including mechanical penetration using appressoria. Appressoria are highly organised enlarged hyphal ends that feature a narrow hyphal strand on the underside known as a penetration peg which penetrates the epidermal cell wall and accelerates inoculation (Howard & Valent 1996; Kavanagh 2005; Mendgen et al. 1996; Sexton & Howlett 2006). Although some pathogenic fungi such as biotrophs are not suitable for mycelium composite applications because they must be in contact with a host plant to survive, non-obligate pathogens are more versatile and can grow and multiply on dead organic matter as well as on living host tissue (Kavanagh 2005). The virulent and aggressive nature of some species allowing them to compete with a living host could make pathogenicity a growth performance indicator able to assist mycelium composite species selection.

Existing fungal taxonomic and association based classification structures could also potentially be used in growth performance predictions to aid mycelium composite species selection. Established taxonomic classifications provide a reference framework of recognisable features, related organisms and useful information about the characteristics of a given species (Webster & Weber 2007). Fungal associations can also be used to describe the preferred growth environment and host-organism relationship shared by groups of fungi (Stamets 2005). Since these pre-existing, well-developed classifications grouped organisms based on morphological, biochemical and ecological similarities, growth performance similarities could also exist, allowing for the prediction of growth performance of multiple species within the same group based on the performance of other group members.

This study investigated simple and resource conservative methods for assessing the viability of fungal species for use in mycelium composite manufacturing applications. It aimed to address these potentially

Table 1. Selected species and associated variables. Compiled from [1]Breitenbach and Kränzlin (1986, 1991, 1995); [2]Stalpers et al. (2004) and Wu et al. (2013).

Identification*	Hyphal Type (mitic)†[1]			Pathogenic‡[2]		Association[2]	Species Variability	Source
	Mono-	Di-	Tri-	Yes	No			
Allomyces arbuscula [Bl]						Water		RMIT
Botrytis cinerea [A]				P				RMIT
Fusarium oxysporum [A]				P		Soil		RMIT
Ganoderma lucidum [Ba]			GBS		x			NGMS
Hypsizygus ulmarius [Ba]	G				x			NGMS
Lichtheimia corymbifera [M]				A		Animal		RMIT
Mucor genevensis [M]						Soil		RMIT
Phytophthora cinnamomi [O]				P		Water		RMIT
Pleurotus citrinopileatus [Ba]							x	NGMS
Pleurotus cornucopiae [Ba]		GB					x	NGMS
Pleurotus djamor [Ba]		GB					x	NGMS
Pleurotus eryngii [Ba]							x	NGMS
Pleurotus ostreatus [Ba]	G				x	Wood	x	NGMS
Pleurotus pulmonarius [Ba]							x	NGMS
Polyporus brumalis [Ba]		GS			x			RMIT
Saksenaea vasiformis [M]				A		Animal		RMIT
Stropharia rugosoannulata [Ba]	G				x			NGMS
Trametes versicolor [Ba]			GBS		x	Wood		NGMS

*Species and phylum, A = Ascomycota, Ba = Basidiomycota, Bl = Blastocladiomycota, M = Mucoromycota, O = Oomycota (Chromista).
†G = generative hyphae (monomitic), GB = generative and binding hyphae (dimitic), GS = generative and skeletal hyphae (dimitic), GBS = generative, binding and skeletal hyphae (trimitic).
‡P = pathogenic to living plants, A = pathogenic to animals or humans.

influential growth factors; determine if they affect hyphal extension rate or growth density, and compare the growth performance of commonly used and non-traditional fungi. Growth performance assessment is more time efficient and cheaper than material property testing and can be used to delimit low-performing species at an early stage in development and identify high-performing species.

Materials and experimental methodology

Fungal cultures

Species selection was systematically based on 1) hyphal type [mono-, di- and trimitic] 2) pathogenicity [pathogenic and non-pathogenic to living plants or animals and humans], 3) fungal association [animal, soil, water, wood] 4) species variability [*Pleurotus* genus] **(Table 1).** Initial selection was based on availability from RMIT culture collections and commercial sources.

Isolates from the RMIT University microbiology culture collection (Bundoora, Australia) were stored cultures under oil on nutrient agar slopes, which were subcultured onto fresh sterile malt extract agar (Neogen, Michigan) plates and their identity verified by technicians. Inverted plates were incubated at 22°C in darkness for 7 days.

Other isolates were purchased from New Generation Mushroom Supplies (Melbourne, Australia) (NGMS). Samples were supplied as mycelial masses growing on wheat grain sealed in plastic bags with filter patches. These isolates were subcultured onto malt extract agar plates and incubated as before.

Media preparation and inoculation

Solid media (hyphal extension rate measured as radial growth)

Malt extract agar (Neogen, Michigan) was prepared as per instructions and autoclaved at 121°C for 15 minutes. Molten agar was poured aseptically into 90 mm petri dishes and allowed to solidify. Isolate cultures were cut into inoculum disks (Ø7 mm) using the blunt base of a sterilised 1.0 mL pipette tip. New pipette tips were used for each species. One single inoculum disk was placed on the edge of each petri dish in contact with the dish wall. Each experiment was conducted with triplicate dishes containing a single inoculum. Triplicate dishes were individually parafilmed, sealed in groups in zip lock bags and incubated at 25°C in darkness for 7 days.

Liquid media (growth density measured as dry weight)

Malt extract liquid media was prepared by mixing malt extract (Morgan's Brewing Co., Yatala) with Milli-Q® water (1 g/10 mL) and autoclaved as described above. The liquid was dispensed as 100 mL aliquots into 250 mL glass jars using a sterile syringe. Inoculum disks were prepared as previously described and suspended in the liquid media. Each experiment was conducted with triplicate jars containing a single inoculum and incubated at 22°C in darkness for 14 days on a Paton Scientific OP3422 orbital shaker at 100 orbits per minute (OPM).

Growth measurements

Hyphal extension rate

Daily radial growth (mm) was measured from the centre of the inoculum disk to the tip of the of the longest hypha. This was completed at the same time each day for consistency for 7 days. This growth period was selected based on preliminary trials which showed the faster species filling a 90-mm petri dish in this time **(Fig. 1a).**

Growth density

End-point dry weight was measured after 14 days of growth. This growth period was selected based on preliminary trials showing this to be the optimal time required for the slower species to have significant enough mass to weigh. The liquid media and mycelial masses were vacuum filtered using grade 1 chromatography paper and an EMD Millipore XX104710 filtration device. Masses were then dried for 48 hours in a 50°C oven and weighed using an OHAUS Explorer analytical balance **(Fig.1b)**.

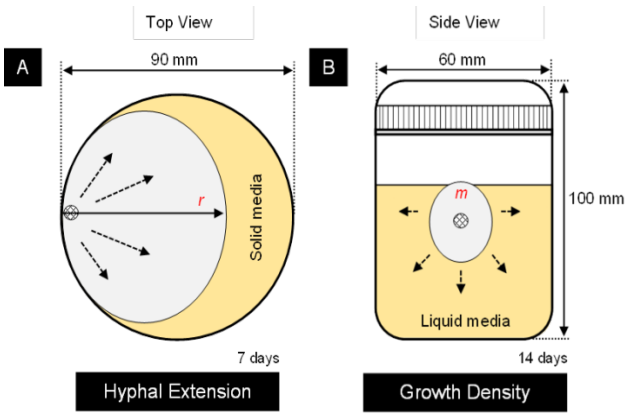

Fig. 1. Mycelial growth measured as a) hyphal extension on agar solid media plates measured radially from the petri dish wall (*r*, mm) over 7 days and b) growth density from broth liquid media measured as 14-day dry weight (*m*, mg). Inoculum disc (cross-hatched, 7mm in diameter) used to inoculate both solid and liquid media.

Data processing and statistical analysis

End-point radial growth and dry weight were statistically analysed in Microsoft Excel and graphed using GraphPad Prism (version 7.02). Data was checked for normality using Kolmogorov-Smirnov test on Minitab (version 16.2) where normal data was $p \geq 0.15$. For data that was normal, ANOVA (Analysis of Variance) was performed, and significant differences were considered at $p \leq 0.05$. Class categories for normal data were generated using letters of comparison based on Tukey's family error rate. Data that was not normal was transformed until normal where indicated, and ANOVA compared. For non-parametric data, Kruskal-Wallis test was conducted where significant differences were considered at $p \leq 0.05$. Class categories for non-parametric data were generated using group membership based on k-means clustering.

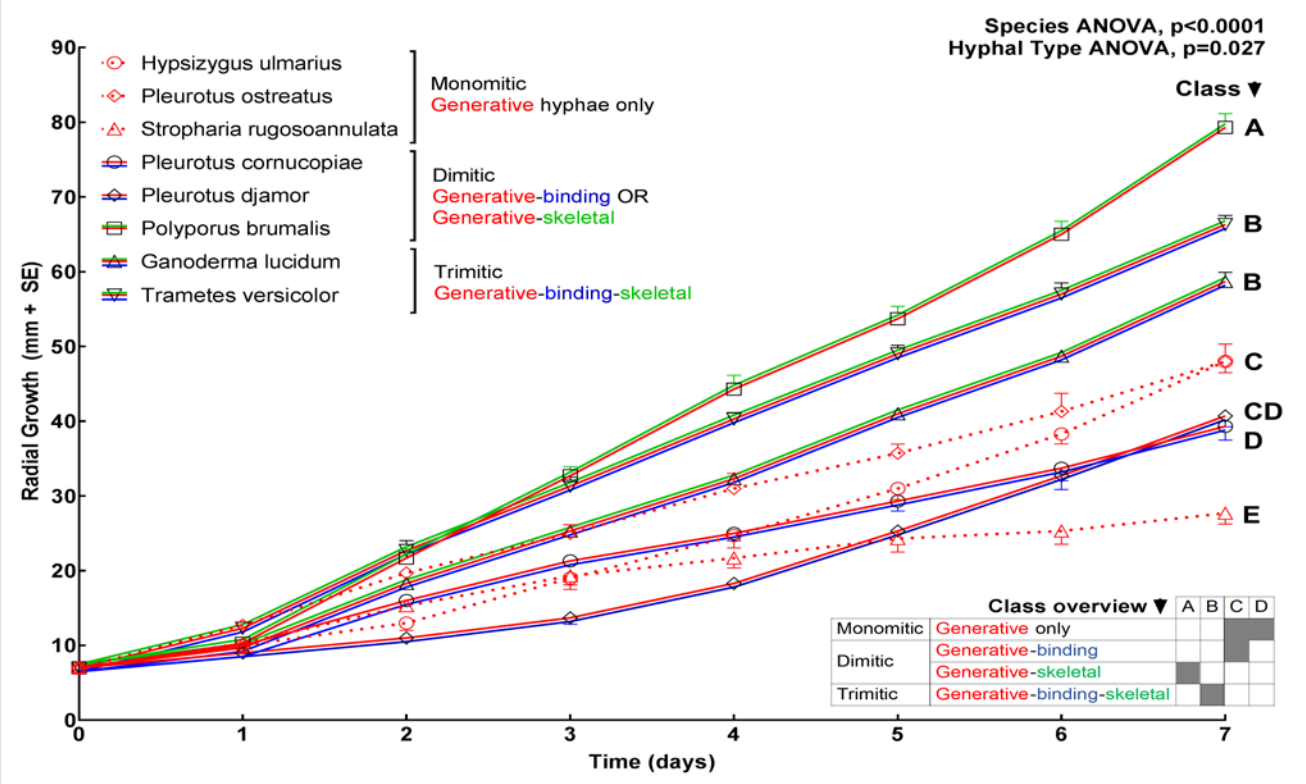

Fig. 2. Hyphal extension rate measured as radial growth (mm + SE) over 7 days for monomitic (generative only, red dotted), dimitic generative-binding (red-blue), dimitic generative-skeletal (red-green) and trimitic (generative-binding-skeletal hyphae, red-blue-green) fungi. Error bars indicate standard error within triplicate sets. Class categories were letters of comparison based on Tukey's family error rate at $p \leq 0.05$ for species dependent ANOVA.

Results

Basidiomycete hyphal types

Significant differences in 7-day hyphal extension measured as radial growth were observed for different hyphal types (ANOVA, p=0.027), although species-specific variation was more significant (ANOVA, p<0.0001). Growth performance varied within, and between the monomitic, dimitic and trimitic groups, however, monomitic species consistently had a slower hyphal extension rate than trimitic species. Dimitic species hyphal extension rate varied greatly depending on whether skeletal or binding hyphae were present in addition to the generative hyphae. Species with generative-skeletal hyphae exhibited a much higher hyphal extension rate than those with generative-binding hyphae **(Fig. 2)** which supported evidence of an inverse relationship between hyphal branching and extension rate.

Monomitic species containing only generative hyphae consistently exhibited low radial growth overall (25-52 mm over 7 days, $\bar{y}$=41 mm) falling exclusively into growth classes C and E. Lowest performing monomitic species, *S. rugosoannulata* (28 mm), (79 mm, highest radial growth) and even *P. ostreatus* and *H. ulmarius* (48 mm) underperformed by almost 40%. Dimitic species exhibited significant variation in radial growth. *P. brumalis* (generative-skeletal hyphae) achieved the highest radial growth overall (class A, 79 mm) while in contrast *P. cornucopiae* and *P. djamor* (generative-binding hyphae) achieved approximately half this value (class C, 39 mm and 41 mm respectively) and performed worse than most monomitic species. Trimitic species consistently achieved high radial growth (57-68 mm, $\bar{y}$=63 mm), falling into growth class B. Highest performing trimitic species *T. versicolor* had the second highest radial growth overall, lagging 16% behind *P. brumalis* **(Fig. 2).**

However, significant differences in growth density measured as 14-day dry weight were not observed for different hyphal types (ANOVA, p=0.198), with significant species-specific variation instead (ANOVA, p<0.0001). There was significant growth performance variation within and between the monomitic, dimitic and trimitic groups with only trimitic species consistently achieving similar results. Monomitic species comprising only of generative hyphae varied significantly, achieving both very low and high dry weights. Dimitic species, while generally high-performing, also varied significantly. The presence of generative, binding or skeletal hyphae appeared not to influence growth density **(Fig. 3).**

Monomitic species had the lowest average dry weight (109 mg), although performance did vary significantly, spanning dry weight classes A-D. Values recorded ranged from 34 mg (*H. ulmarius*) to 238 mg (*P. ostreatus*) (7 times difference) which were respectively the lowest and second highest values recorded overall. Dimitic species had the highest average dry weight (159 mg), but performance varied from 105 mg (*P. brumalis*,

generative-skeletal hyphae) to 251 mg (*P. djamor*, generative-binding hyphae) which was the highest dry weight recorded overall (over 2 times difference, classes A, B, C). Trimitic species had medium average dry weight (138 mg), falling in classes B and C, and ranging from 119 mg (*G. lucidum*) to 165 mg (*T. versicolor*) **(Fig. 3).**

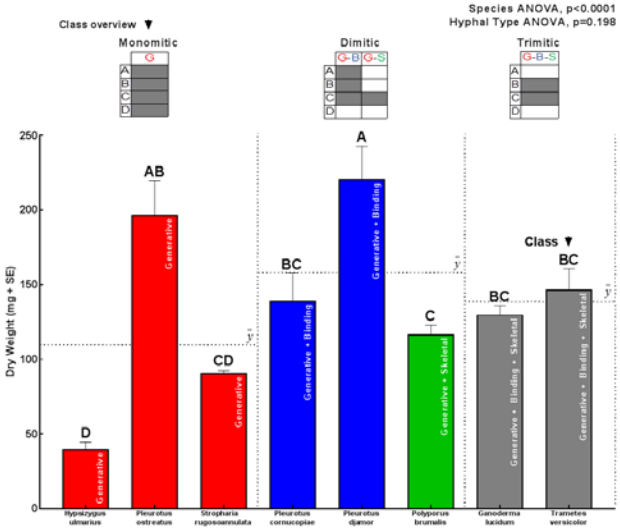

Fig. 3. Growth density measured as 14-day dry weight (mg + SE) for generative monomitic (G, red), dimitic generative-binding (G-B, blue), dimitic generative-skeletal (G-S) (green) and trimitic (G-B-S, grey) fungi. Class categories were letters of comparison based on Tukey's family error rate at p≤0.05 for species dependent ANOVA.

Pathogenicity

The 7-day hyphal extension measured as radial growth was not significantly different between pathogenic and non-pathogenic species (ANOVA, p=0.079). Instead, species-specific variation was significant (ANOVA, p<0.0001). Growth performance varied significantly within the pathogenic and non-pathogenic groups, with group representatives in most classes which ranged from slow (10-50 mm) to fast (50-90 mm). The outlier, *S. rugosoannulata*, underperformed and was the slowest species **(Fig. 4).**

Pathogenic species (47-84 mm, ȳ=67 mm, classes A-E) experienced higher average radial growth performance than non-pathogenic species (25-83 mm, ȳ=55 mm, classes A-F). However, the highest performing pathogenic species *S. vasiformis* (82 mm) was only marginally faster than the highest performing non-pathogenic species *P. brumalis* (79 mm), and the performance of most non-pathogenic species investigated was better than or comparable to at least one pathogenic species. The slowest pathogenic species, *P. cinnamomi* (50 mm) experienced radial growth only marginally faster than the non-pathogenic *P. ostreatus* and *H. ulmarius* (both 48 mm) and was outperformed by two-thirds of all species. The slowest non-pathogenic species, *S. rugosoannulata*, was almost twice as slow as the slowest pathogenic species (28 mm) **(Fig. 4).**

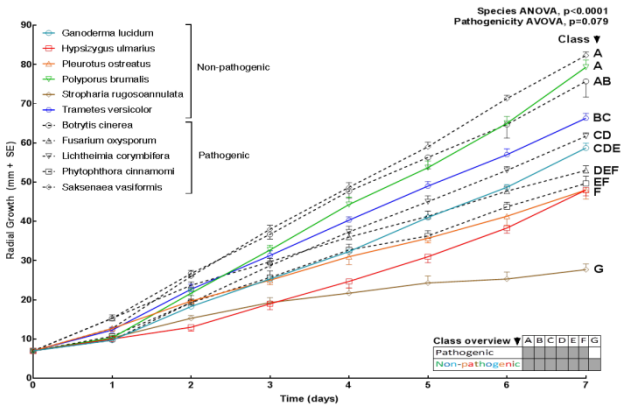

Fig. 4. Hyphal extension rate measured as radial growth (mm + SE) over 7 days for pathogenic (black dotted) and non-pathogenic fungi (coloured) fungi. Class categories were letters of comparison based on Tukey's family error rate at p≤0.05 for species dependent ANOVA.

Differences in growth density data measured as 14-day dry weight had borderline significance between pathogenic and non-pathogenic species (Non-parametric, Kruskal-Wallis, p=0.051), but variation determined by individual species was far more significant (Kruskal-Wallis, p=0.001). Growth performance was comparable within and between pathogenic and non-pathogenic groups except species-specific outliers like *H. ulmarius* and *F. oxysporum*.

Pathogenic species had higher overall average dry weight (89-316 mg, ȳ=172 mg) than non-pathogenic species (34-160 mg, ȳ=120 mg). Pathogenic *F. oxysporum*, as an outlier, experienced the highest dry weight (316 mg), however, this was not characteristic of pathogenic species with most performing approximately 50-70% worse than *F. oxysporum*. Non-pathogenic outliers included *P. ostreatus* (197 mg) and *H. ulmarius* (40 mg), but dry weight of pathogenic and non-pathogenic fungi was comparable for all other species with very similar performance noted between several fungi of opposing pathogenicity status. Non-pathogenic *H. ulmarius* had the lowest dry weight (40 mg), but this was also not characteristic of non-pathogenic species with most performing approximately 2-5 times better **(Fig. 5).**

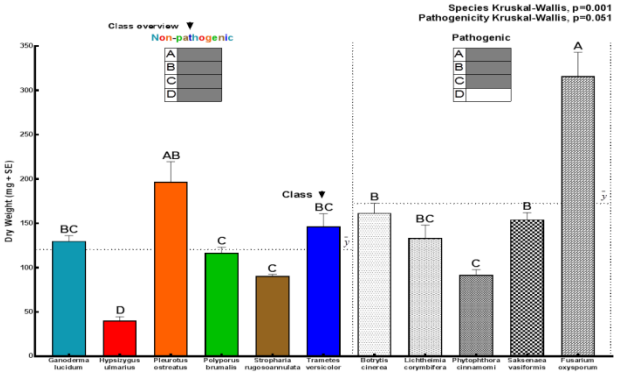

Fig. 5. Growth density measured as 14-day dry weight (mg + SE) for non-pathogenic (coloured) and pathogenic (greyscale) fungi. Class categories were letters of comparison based on cluster membership at p≤0.05 for species dependent k-means clustering.

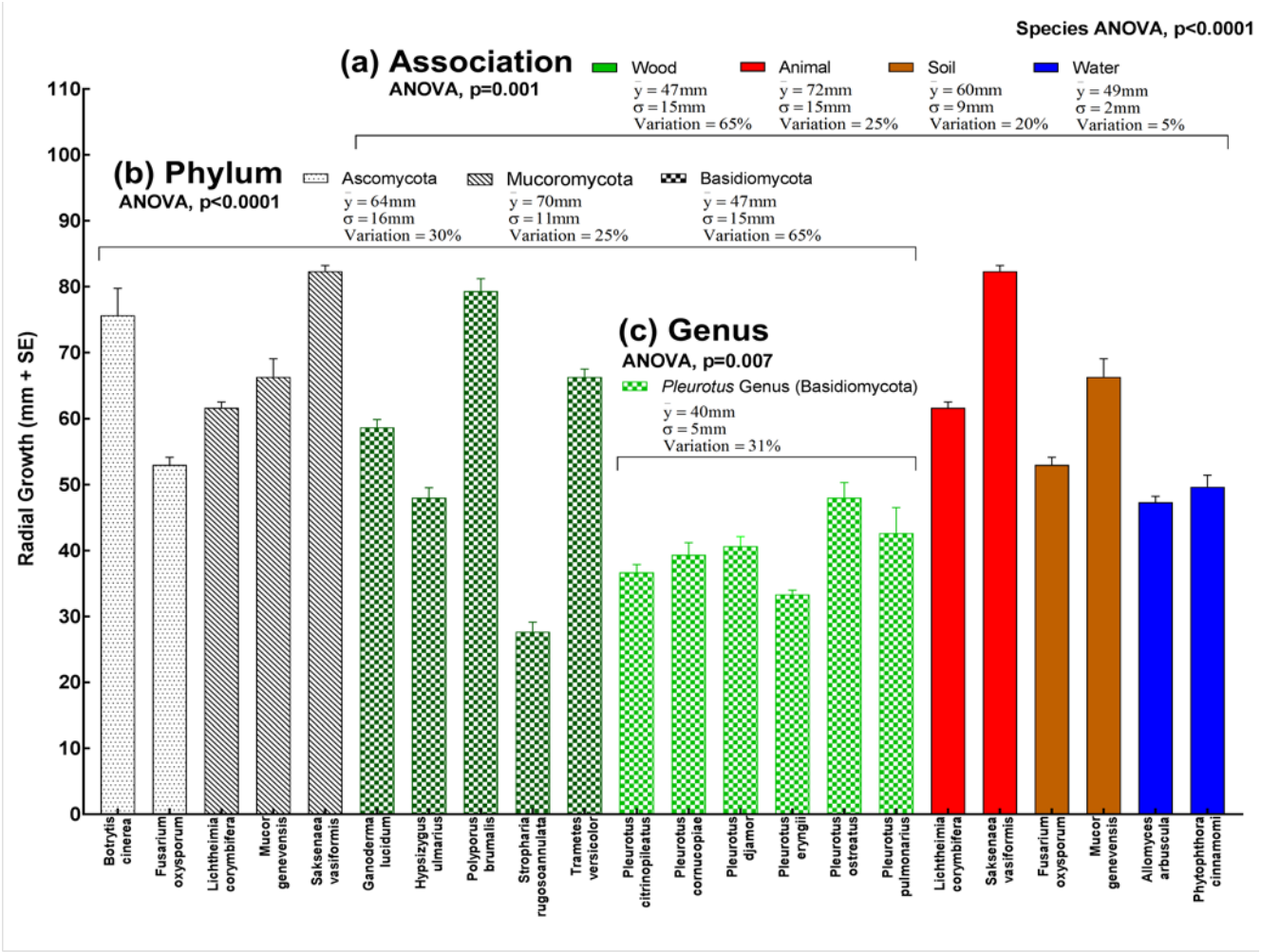

Fig. 6. Hyphal extension measured as 7-day radial growth (mm + SE) for (a) fungal association, (b) phylum and (c) species-specific variation within the same genus (*Pleurotus*). Colouring: animal (red), soil (brown), water (blue) and wood (green) fungal associations. Shading: Ascomycota (dotted), Basidiomycota (checkered) and Mucoromycota (diagonally striped) phyla. *Pleurotus* genus highlighted (light green checkered). The mean ($\bar{y}$) and standard deviation (σ) of each data set is included in addition to the percentage variation between minimum and maximum values.

Taxonomic and fungal association based classifications

Most significant differences in 7-day hyphal extension measured as radial growth were observed between different species and phyla (ANOVA, $p<0.0001$). Significant differences were also observed between the radial growth of fungi of different associations (ANOVA, $p=0.001$) with least significant differences observed between fungi within the *Pleurotus* genus (ANOVA, $p=0.007$) **(Fig. 6).**

Wood-associated fungi experienced the highest variation in radial growth based on association (σ=15 mm, variation=65%) **(Fig. 6a)** with a sample size (n=11) sufficiently large to demonstrate the heterogenous nature of fungi and preclude a correlation between the fungal association and hyphal extension rate. Although a micro trial (n = 2) of water-associated fungi displayed low variation (σ = 2 mm, variation = 5%), other micro trials conducted on the animal- (σ = 15 mm, variation = 25%) and soil- (σ = 9 mm, variation=20%) associated fungi demonstrated significant variation and demonstrated the futility of further trials.

Comparison by phylum **(Fig. 6b)** yielded similar results with Basidiomycota synonymous with wood associated fungi in this case. The significant variation (σ = 15 mm, variation=65%) present within the large sample of Basidiomycota (n = 11) coupled with micro trials of Ascomycota (n = 2) (σ = 16 mm, variation = 30%) and Mucoromycota (n = 3) (σ = 11 mm, variation = 25%) demonstrated significant variation present within and between phyla and the insignificance of phylum on radial growth.

A final investigation of genetically similar fungi within the *Pleurotus* genus (n = 6) (σ = 5 mm, variation = 31%) **(Fig. 6c)** confirmed the significant species-specific variation in radial growth between fungi and the absence of a relationship between traditional taxonomic and fungal association based classifications and hyphal extension growth performance.

Most significant differences in 14-day growth density measured as dry weight were observed between different species (including genetically similar species within the *Pleurotus* genus) (ANOVA, $p<0.0001$). Significant

differences were also observed in the dry weight of fungi between phyla (ANOVA, p=0.001) with least significant differences observed between fungi of different associations (ANOVA, p=0.005) **(Fig. 7).**

Wood-associated fungi experienced the highest association based variation in growth density (σ=55 mg, variation=82%) **(Fig. 7a)** with a sample size (n=11) sufficient to suggest that fungal association had little impact on growth density. Although a micro trial (n = 2) of animal-associated fungi did show lower variation (σ = 15 mg, variation=14%), most micro trials including those of water- (σ = 230 mg, variation = 77%) and soil- (σ = 118 mg, variation = 53%) associated fungi also demonstrated significant variation, making further trials redundant.

Comparison by phylum **(Fig. 7b)** yielded similar results with Basidiomycota synonymous with wood associated fungi for the species examined. Although, a micro trial of Mucoromycota (n=3) indicated some uniformity (σ = 11 mg, variation = 14%), significant variation (σ = 55 mg, variation = 82%) within the extensively sampled Basidiomycota (n = 11) coupled with a micro trial of Ascomycota (n = 2) (σ = 109 mg, variation = 49%) demonstrated significant variation present within and between phyla and the lack of influence of this factor on growth performance.

A final investigation of genetically similar fungi within the *Pleurotus* genus (n = 6) (σ = 65 mg, variation = 72%) **(Fig. 7c)** verified the significant species-specific variation in dry weight between fungi and the lack of correlation between traditional taxonomic and fungal association based classifications and growth density.

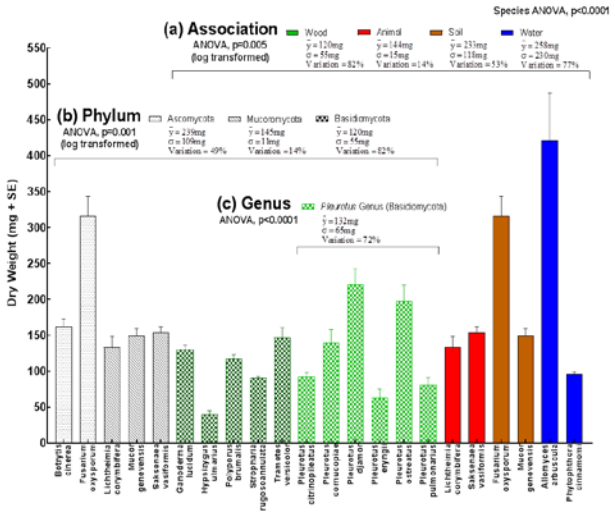

Fig. 7. Growth density measured as 14-day dry weight (mg + SE) for (a) fungal association, (b) phylum and (c) species-specific variation within the same genus (*Pleurotus*). Colouring: animal (red), soil (brown), water (blue) and wood (green) fungal associations. Shading: Ascomycota (dotted), Basidiomycota (checkered) and Mucoromycota (diagonally striped) phyla. *Pleurotus* genus highlighted (light green checkered). The mean ($\bar{y}$) and standard deviation (σ) of each data set is included in addition to the percentage variation between minimum and maximum values.

Performance overview

Significant variation in hyphal extension (28-82 mm) and growth density (40-421 mg) was present in the fungi assessed. *S. vasiformis* experienced the fastest hyphal extension over 7 days measured as radial growth (82 mm) overall, and *A. arbuscula* achieved the highest growth density over 14 days measured as dry weight (421 mg) **(Fig. 8).**

Other fungi (*B. cinerea*, *M. genevensis*, *P. brumalis* and *T. versicolor*) experienced radial growth or dry weight within 20% of values attained by the highest performing species. A further 25% of fungi (*F. oxysporum*, *G. lucidum*, *L. corymbifera* and *P. cinnamomi*) attained radial growth or dry weight within 20-40% of the highest performing species while the remaining 50% of fungi (*H. ulmarius*, *P. citrinopileatus*, *P. cornucopiae*, *P. djamor*, *P. eryngii*, *P. ostreatus*, *P. pulmonarius*, *S. rugosoannulata*) performed at least 40% worse than the highest performing species **(Fig. 8).**

The poorest growth performance was exhibited by *S. rugosoannulata* (28 mm hyphal extension) and *H. ulmarius* (40 mg dry weight). Medium (60-80% performance) and high-performing (top 20% performance) fungi attained either high radial growth or high dry weight but never both. Some low-performing fungi (0-60% performance) had more proportional radial growth and dry weight (*P. cornucopiae* and *P. ostreatus*) but overall high radial growth and high dry weight were mutually exclusive in species assessed **(Fig. 8).**

Discussion

Daily radial growth measurement on solid media was an economical, efficient and effective method for assessing the hyphal extension rate of fungi. Trends in individual hyphal extension acceleration and deceleration were visible over as little as 7 days, and growth performance comparison between fungi was also possible for this period. This two-dimensional metric, combined with a three-dimensional growth density assessment derived from fungi grown in liquid media, comprehensively described fungal growth potential and could be used to delimit low-performing species at an early stage in development and help identify high-performing species.

Growth performance varied significantly and arbitrarily meaning that for optimal species selection, fungi need to be assessed on an individual basis to establish their suitability for composite manufacturing applications. Hyphal growth involves cell wall extension and biosynthesis of wall components utilising chitin synthase isozymes with different kinetic parameters (K_M values) that vary in type and number by species (Carlile *et al.* 2001). Hyphal extension rate is also related to hyphal extension zone and colony peripheral growth zone dimensions which vary not only by species but by strain (Carlile *et al.* 2001).

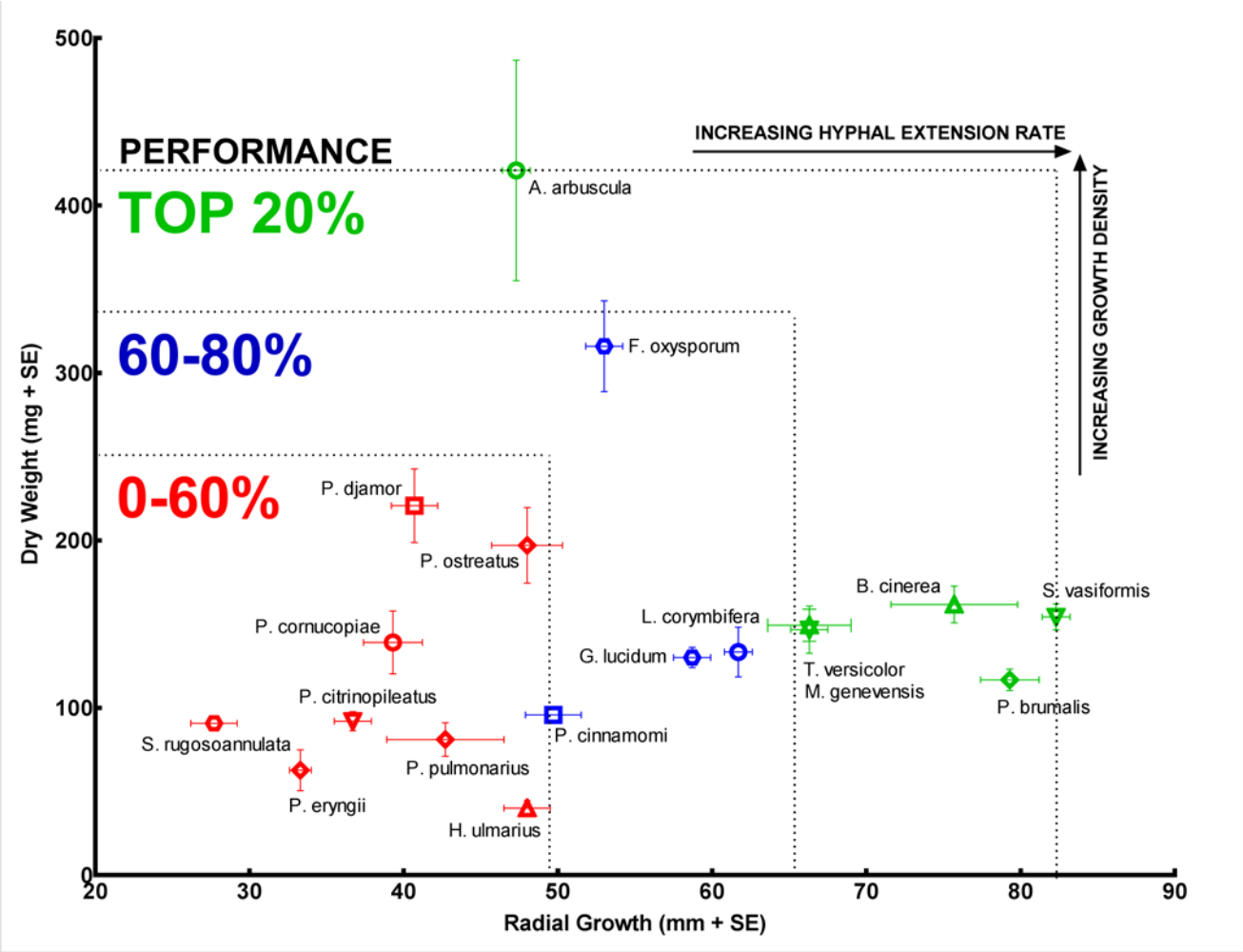

Fig. 8. Hyphal extension (7-day radial growth in mm) versus growth density (14-day dry weight in mg) for all fungi assessed grouped by percentage performance (low 0-60% - red, moderate 60-80% - blue, top 20% - green).

Hyphal extension rate and branching were inversely related for basidiomycetes in this study, with dimitic species containing sparsely branched or unbranched skeletal hyphae exhibiting much higher hyphal extension rates than dimitic species containing highly branched binding hyphae. However, an inverse relationship between growth density and branching of basidiomycetes was absent. Hyphal extension rate and branching are also inversely related for ascomycetes (Robinson & Park 1966) and the hyphal growth unit (G), which is a property of the mycelium that is mathematically linked to other hyphal and colony growth parameters (Kotov & Reshetnikov 1990), increases as branching becomes sparser (Prosser 1995). The effect of branching on dry weight (growth density) for basidiomycetes has not been extensively studied, but branching is known to increase the surface area of colonies and mediate hyphal fusion events which aid nutrient assimilation and exchange between hyphae of the same colony (Harris 2008).

Pathogenic fungi, which rapidly invade and colonise host material utilising a combination of specialised toxins, cellular process subversion and mechanical force (Kavanagh 2005; Sexton & Howlett 2006) absent in the less aggressive saprotrophic species, were expected to exhibit improved growth performance. However, no consistent or significant growth performance improvement was present in these fungi. Pathogenic species are inherently more dangerous than non-pathogenic species with their infectious nature making them more difficult to render inert and hence safe materials harder to produce. Significant risks are also present during their manufacture for either plants or animals (including humans) exposed to the manufacturing process. As such, with no significant growth performance improvement present among these species, their use should be excluded from mycelium composite manufacturing.

It is not known how variation in the substrate and environmental conditions would affect the growth performance of the fungi investigated as this varies on a species-specific basis. However, water-, animal- and soil-associated fungi provided no notable growth performance improvement and since most composite substrates are starch, cellulose or lignin-based (Haneef *et al.* 2017; Holt *et al.* 2012; López Nava *et al.* 2016; Pelletier *et al.* 2013; Travaglini *et al.* 2013), wood-associated fungi would be enzymatically best suited to digest these complex carbon

sources. *T. versicolour* and *P. brumalis* were the highest performing wood-associated fungi, achieving growth performance within the top 20% of species overall. This supports their use in mycelium composites applications over other traditionally popular species including *G. lucidum* (Haneef *et al.* 2017; Holt *et al.* 2012; Travaglini *et al.* 2013) and *P. ostreatus* (Haneef *et al.* 2017; He *et al.* 2014; López Nava *et al.* 2016).

Growth performance assessment is of critical importance due to the inherently slow nature of mycelium composite manufacturing which take days to grow (Haneef *et al.* 2017; Holt *et al.* 2012; López Nava *et al.* 2016; Pelletier *et al.* 2013; Travaglini *et al.* 2013) when compared to traditional plastic manufacturing processes which require only minutes or hours to produce (Allen 2012). However, mycelium composite manufacture is much less energy intensive than plastic manufacturing and utilises agricultural waste materials to produce low density, biodegradable products rather than petroleum derivatives (Holt *et al.* 2012; López Nava *et al.* 2016; Pelletier *et al.* 2013; Travaglini *et al.* 2013). This makes mycelium composites cost competitive with plastics but significantly more environmentally sustainable (Holt *et al.* 2012; Pelletier *et al.* 2013; Travaglini *et al.* 2013) with production time able to be reduced though informed species selection governed by growth performance screening methodologies such as those proposed in this study.

Conclusion

This study found the assessment of hyphal extension rate measured as radial growth and growth density measured as dry weight to be a simple, effective and resource conservative method for evaluating the viability of fungi for mycelium composite manufacturing. Hyphal types present, pathogenicity and traditional classification structures were not influential growth factors, with fungal growth performance highly variable both in terms of hyphal extension rate and growth density. However, growth performance can be rapidly and inexpensively determined through methods such as those outlined in this study. This makes initial growth performance screening prior to more expensive and complicated testing possible. *T. versicolour* and *P. brumalis* were the most suitable species assessed in this study based on growth performance and enzymatic compatibility with typical mycelium composite substrates. This supports their use over other traditionally used species such as *P. ostreatus* and *G. lucidum*.

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