What is the contribution of autumn senescent leaves to the aeromycota in an urban environment?

Peter J. Irga

Margaret D. Burchett

Gabe O'Reilly

Fraser R. Torpy

Plants and Environmental Quality Research Group, School of the Environment, Faculty of Science, University of Technology Sydney, PO Box 123, Broadway, NSW 2007, Australia

e-mail: Fraser.Torpy@uts.edu.au

Phone: +61 2 9514 4150

Fax + 61 2 9514 4079

Abstract

Street trees in urban areas are often deciduous and drop leaves during autumn. This investigation aimed to assess the potential contribution of senescent leaves to the diversity of airborne fungal propagules during the season of autumn. The senescent leaves of five deciduous tree species were subject to a manipulative experiment in which their phyllospheric fungi were aerosolized, and air samples taken to document the spores derived from leaf material. Aerosolized fungi were compared with the fungi detected from direct leaf of the phyllosphere. Thirty-nine genera were identified across the plant species sampled, of which twenty-eight genera were present in the corresponding air samples. Significant differences were observed amongst the fungal genera growing on the leaves of the different trees, however few differences were found in the composition of fungal spores that were aerosolized. The dominant genera that were aerosolized were: *Penicillium*, *Cladosporium*, *Alternaria*, *Chaetomium*, *Botrytis* and *Trichothecium*. As these fungal genera are commonly identified in autumn air samples in other studies, it is likely that the phyllospheric fungi present on deciduating leaves contribute to the aeromycota of urban areas.

Keywords: Airborne fungi; phylloplane; urban; aeromycota; autumn; leaf surface fungi

1 Introduction

Airborne fungal propagules constitute a large proportion of the biological matter in the air (Bauer et al. 2008). When inhaled, these spores, hyphal fragments and desiccated yeast cells may have serious health implications (Tham et al. 2014), leading to clinical conditions ranging from temporary allergic reaction, to systemic disease (Crook & Burton 2010, Pfaller et al. 2006). Many studies have found seasonal variations in the density and composition of airborne fungal propagules. These seasonal variations are commonly attributed to meteorological influences such as humidity, temperature and wind speed (Sabariego et al. 2000). However, it is possible that there is an association between the phenology of airborne fungal behaviour with seasonal changes that affect the sources or substrates of the fungi. Evidence to support this comes from Mitakakis & Guest (2001), who observed that airborne spore levels in the city of Melbourne, Australia were maintained at high levels in autumn despite relatively low humidity and temperatures. The authors (Mitakakis & Guest 2001) hypothesised that this may have been due to senesced leaves acting as a substrate and reservoir for fungal growth.

Whilst it is well known that fungi growing on plant material produce spores which may aerosolize and then colonise other plants (Brown & Hovmøller 2002, Pepeljnjak & Šegvić 2003), the contribution of fungi from the phylloplane of senescent leaves to the spores that are present in the air has never been assessed. Additionally, whilst some attention has been focused on how the immigration of atmospheric fungal spores can influence the microbial composition of the phylloplane (Zak 2002), little attention has been dedicated to how the microbial population of the phylloplane may affect the assemblage of spores released into the atmosphere — and those that we breathe.

Levetin and Dorsey (2006) conducted a study to associate leaf surface fungi from two tree species to matched air samples from the same location. The authors suggested that some of the fungal species from living leaves had the potential to become contributors to the air spora. If this was the case, it is probable that decaying leaves would also contribute to spores in the air, especially during autumn when fallen senescent leaves make up an ideal environment for many saprophytic microorganisms.

The decay of senesced leaves may be of particular significance in an inner urban or city centre environment, where deciduous street trees could be the only substantial source of detritus in the area. Deciduous trees are often preferred in inner urban environments as they allow light through ‘urban canyons’ during winter, are relatively resistant to pollution, and are able to be easily ‘sculpted’ (City of Sydney 2003). Sydney, Australia is a large urban centre that utilises street trees following this design. Many street trees within the City of Sydney local governmental area are autumn-deciduous and drop leaves mainly onto fully paved surfaces. Furthermore, Sydney is a city with a relatively low biodiversity within the deciduous urban forestry: London plane trees (*Platanus* x *acerifolia*) have been planted along > 20% of streets in the City of Sydney local governmental area (LGA), representing 9.5% of total trees within this area, and make up the overwhelming majority of all deciduous trees in the area (City of Sydney 2013). If these trees support a constrained range of phylloplane fungi, it is possible that the low biodiversity of street trees in Sydney may potentially yield a limited diversity of aerosolized fungi. High concentrations of relatively few species could in turn pose a threat to human health if those species were allergenic or pathogenic. Any such health hazard would be further localized if a fungal species that has been associated with adverse human health outcomes had specificity for a particular tree species which was prevalent in an area.

In the current study, a manipulative experiment was used, to estimate the potential contribution of phyllospheric fungi on deciduating and decaying leaves to the aeromycota of their proximal atmospheres. The specific research aims were to identify the phylloplane fungi on senesced autumn leaves from the five most prevalent deciduous street tree species of Sydney; and assess the contribution of the phylloplane fungi to the diversity of spores that are aerosolized from the fallen leaves.

2 Materials and Methods

2.1 Study area

The study was conducted in the inner city of Sydney, Australia (Fig. 1). Sample trees were distributed across the LGA as shown in Figure 1. All sampled trees were more than 200 m in distance from the nearest tree of the same species.

2.2 Plant material

The investigation was made using abscised leaves from the five most prevalent deciduous tree species in the urban Sydney streetscape. Trees were identified in collaboration with Karen Sweeney, the Senior Arborist at the City of Sydney Council. The sampled species were:

- *Celtis australis;* European hackberry
- *Platanus* x *acerifolia;* London Plane tree
- *Populus nigra* Lombardy Poplar
- *Robinia pseudoacacia* var. 'Frisia'; Golden Robinia
- *Triadica sebiferum* (syn. *Sapium sebiferum*: Esser, 2002); Chinese Tallow tree

Leaves from all tree species were collected between late May and early June 2014 (late autumn to early winter) when leaf drop was at its peak. All trees were over 5 m in height, and had been growing *in situ* for at least a full seasonal cycle. Leaves were collected from 1–3 m above ground level from 4 individual trees per species. To reduce contamination from soil-borne microorganisms, leaves were not collected from the ground, but were collected after they had either fallen by natural abscission when lightly touched, or when gently shaking the branch. We recognise that there are potentially differences amongst the various locations within the canopy with respect to phyllospheric fungi (Osono & Mori 2004); these differences were randomized in this study by collecting senesced leaves that were in the early stages of abscission (about to fall from all parts of the trees).The leaves were placed immediately into sterile plastic sealable bags, and were transferred to experimental chambers on the day of collection. The volume of leaves collected was approximated so as to cover a 20 x 30 cm area when spread out. Leaf surface area rather than mass was compared across treatments as it was considered to be more relevant to streetscape scenarios (leaves falling onto pavements), and it allowed consistent comparisons of leaf surface areas amongst sample species.

2.3 Treatments

For each sample, leaves were arranged in sampling trays (20 x 30 cm) so as to cover the surface area in a single layer. Trays were placed in separate, pre-sterilized (70% ethanol), sealed Perspex chambers (216 L), for a total of 21 days, during which 3 successive sets of fungal samples were taken as explained below. Empty chambers were sampled before and after the treatments to ensure that neither the chamber nor the atmosphere were contributing to the fungi detected.

A period of 21 days was used as the experimental test period, as this encompassed the maximum length of time leaves would normally be left on the street in a well-maintained city. In the City of Sydney leaves are removed “when required” by street cleaners (K. Sweeney — Pers. Comm.), depending on the density of human traffic in the area; this interval can be as frequent as daily, in densely populated areas.

Chamber temperatures were regulated at 22±0.1°C, and the treatments were subjected to a 10/14h (day/night) photoperiod at a light intensity of 50±5 µmol PAR $m^{-2}s^{-1}$, to represent Sydney diurnal light regimes during autumn and winter.

As Sydney has a variable climate in autumn, with periods of both high and low rainfall, separate trays of leaves were maintained at two humidity levels:

a) *100 % humidity*: 150 mL water was carefully added to each sampling tray to replicate conditions of a running city gutter in autumn, when rainfall in Sydney for that season is high (In the year 2014, actual total rainfall for autumn ranged from 39 to 68 mm per month; BoM, 2014). The added water maintained saturated humidity levels in the chambers throughout the test period.

b) *45–55% humidity*: No moisture was added to the trays or chambers. This treatment reflected the situation where fallen leaves lie on dry pavements. The treatment also represents the low humidity levels often experienced in Sydney during late autumn and early winter, when fallen leaves are at their most prevalent.

Both leaf surface and simultaneous chamber air samples were taken at days 7, 14 and 21 after placement in the experimental chambers. We did not attempt to measure succession in this experiment, rather, we randomized the effects of succession by taking three samples over the course of the experiment: at day 7, 14 and 21, and summing the results.

2.4 Direct phylloplane assessment

As leaves within an urban environment tend to fall onto footpaths, roads and other sealed concrete surfaces where contamination from substrate fungi would be limited, we only considered the contribution of phyllospheric fungi to the decay of the leaves.

On each chamber sampling occasion, subsamples of leaves were removed (10 ± 1 cm^2 of leaf area per chamber) and fungi were identified via direct assessment, as follows: Fungi were qualitatively sampled from leaf surfaces using the cellotape leaf impression technique (Wickremasinghe et al. 1985), staining with lactophenol cotton blue. Fungi were examined using light microscopy, and identified to genus level using published keys (Agrios 2005, Alexopoulos et al. 1996, De Hoog et al. 2000, Ellis et al. 2007, Klich & Pitt 1988, Larone 2002). Differences between the abaxial and adaxial surfaces of the leaves were randomised by arranging the leaves in the chambers in random alignments, as they would be expected to fall on a city street. The entire 10 cm^2 of leaf area was analysed (at 1 cm^2 cellotape leaf impression per observation, thus approximately 100 observations per sample), and fungal colonisation was quantified as the proportion of total observations occupied by a specific fungus for each sample. These values were then summed, and have been presented as proportions of total spores identified to enable comparisons with air samples.

2.5 Air samples from decaying leaf matter

Prior to the removal of the chamber air samples, 100 mm diameter fans mounted within the chambers were used to aerosolize spores. Fans were turned on for 1 minute, generating a wind velocity of 1.5 m/s at the surface of the leaves, after which samples were taken immediately. Air samples of 5 L were taken from each chamber utilising a Reuter Centrifugal air sampler (RCS; Biotest Diagnostics Corporation, Denville, New Jersey, USA). Sabouraud's dextrose agar (SDX; Biotest AG, Germany) was used as the growth medium. This sample size produced 50–300 colonies on the strips. The sampler was sealed in the chambers during sample collection to eliminate drawing outside air into the chambers.

After sampling, agar strips were aseptically removed from the RCS, re-sealed in their plastic sleeves and incubated in the dark at 23^{o}C for 7 days. If colony development was inadequate after this period, strips were re-incubated until adequate colony growth occurred (up to 21 days). Fungi were sampled from the strips using cellotape and stained with lactophenol cotton blue. Colonies were identified to genus level using colonial and microscopic morphology, utilising the published keys as above. Final colony counts were expressed as proportions of total species identified, since 'absolute' spore counts would not be representative of the airborne spore density that might be present in an open atmosphere surrounding the leaves *in situ*.

2.6 Data analysis

Comparisons of univariate variables across treatments were made with general linear model ANOVA (SPSS v20; IBM Corp. 2011). Data were transformed where necessary to improve homogeneity of variances. As per

Torpy et al. (2012), differences in fungal community structure amongst treatment groups were assessed using multivariate analysis of similarities (ANOSIM), using Euclidean distance similarity matrices. When differences were identified between treatment groups, similarity percentages analysis (SIMPER) was utilised to identify the fungal genera that best represented the differences. Multivariate data analyses were performed using PRIMER v6.1.6 (Primer-E Ltd, 2006).

3 Results

The results presented in Tables 1 to 5 demonstrate that the phylloplanes of the sampled plant species were colonised by dense and diverse fungal communities. However, not all fungi became aerosolized; with air samples showing significantly lower numbers of genera across all treatments (ANOVA: d.f = 1, P = 0.01). A total of 39 genera were identified across the plant species sampled, of which 28 genera were present in the corresponding air samples.

The genera most prevalent in the air samples were *Penicillium*, *Cladosporium*, *Alternaria*, *Chaetomium*, *Botrytis* and *Trichothecium*. Few serious human pathogens were observed in concentrations so as to be of concern to health; although pathogens like *Aspergillus fumigatus* were present in a small number of samples; however they were never detected in high concentrations.

Yeast cells were identified far more frequently in air samples than from leaf impressions. This was possibly due to the yeasts being present in a desiccated state in the phyllosphere, where identification from the impressions would be extremely difficult, but would be simple after culture from the air samples.

Under the lower humidity condition, which reflected conditions commonly experienced during early winter months in Sydney, the diversity (number of genera detected) of both the phylloplane fungi and those that became aerosolized, were significantly lower than the high humidity samples (both P = 0.000).

Furthermore, the community structure of the fungi of the lower humidity treatments also differed to the high humidity treatments (ANOSIM: Global R = 0.521, P = 0.01); with many of the plant pathogens like *Curvularia*, *Rhizopus*, *Pithomyces*, *Phialophora*, *Didymella*, *Bipolaris*, *Periconia*, and *Geotrichum* detected exclusively in the high humidity treatments.

Fungal community structures identified from the leaf phylloplane differed compared to those identified in their respective air samples (ANOSIM: Global R = 0.785, P = 0.01). Whilst the species composition in the air samples clearly originated from the fungal community from the leaf samples, the pairwise differences between treatments showed a consistent pattern: all air samples were dominated by easily aerosolized spores, while the fungi present on the leaf samples were more diverse. SIMPER analysis indicated that these differences were driven by *Phialophora* and *Cladosporium* being more prevalent in the air samples than the leaf samples, and *Alternaria* were predominantly detected from the leaf samples, but rarely from the chamber air samples.

Differences in fungal community assemblage across plant species were observed, at both the high (ANOSIM: Global R = 0.285, P = 0.01) and low humidity treatments (ANOSIM: Global R = 0.175, P = 0.01). At the higher humidity level, fungal community structures identified in the leaf phylloplane samples differed amongst all tree species (ANOSIM: Global R = 0.285, P=0.001), except for comparisons between *R. pseudoacacia* and *T. sebiferum* (R = 0.088, P = 0.069), and *P. nigra* and *C. australis* (R = 0.021, P = 0.258). In most cases, *Alternaria* was the primary taxon differentiating amongst the treatments, contributing to 29.87–30.90 % of the overall between-group variance (average proportional frequency across leaf samples = 19.4, with *C. australis* and *P. nigra* both recording lower frequencies of *Alternaria* than the other plant species.). The other taxa that contributed more than 10% of the between group differences were *Cladosporium*, *Aureobasidium pullulans*, *Trichothecium* and *Ustilago*.

Comparisons of the community structure of the fungi aerosolized from these leaf samples yielded no significant differences, except for the fungi aerosolized from *P. nigra* compared to *Platanus x acerifolia* (R = 0.261, P =

0.004) . These differences were characterised by increased *Trichothecium* and *Cladosporium* from the *P. nigra*, compared to high levels of *Alternaria*, *Chaetomium* and *Penicillium* derived from the *Platanus x acerifolia*.

At the lower humidity level, fungal community structures identified in the leaf phylloplane samples differed across all tree species (ANOSIM: Global R = 0.175, P = 0.001), except for comparisons between *Platanus x acerifolia* and *C. australis* (R=0.028, P = 0.19). Variations in the relative densities of *Penicillium*, *Cladosporium* and *Alternaria* generated these differences. Comparisons of the community structure of the fungi aerosolized from these leaf samples yield no significant differences, except for the fungi aerosolized from *P. nigra* compared to those from *Platanus x acerifolia* (R = 0.198, P = 0.008), *R. pseudoacacia* (R = 0.155, P = 0.005) and *C. australis* (R = 0.141, P = 0.008). These differences were mainly driven by the higher concentrations of *Trichothecium* on the *P. nigra.*

4 Discussion

The most frequent genera detected in this study were similar to those reported from previous research conducted during autumn in urban Sydney (Irga et al. 2014), with *Penicillium*, *Cladosporium* and *Alternaria* common. Furthermore, the community of aerosolized spores in this study is similar in diversity and relative frequencies of species encountered in outdoor autumn samples identified by Torpy et al. (2013). It was evident from this work that *Alternaria* and *Cladosporium* have a wide host plant range, having been found in high frequencies in all plant phylloplanes examined. This corresponds to previous research conducted in rural New South Wales, where *Alternaria* was isolated from all plant materials sampled (Mitakakis et al. 2001). Similarly, *Cladosporium* spp. appears to be the most predominant outdoor airborne fungal spore type in eastern Australia, occurring in 75% of all samples in urban Sydney (Irga et al. 2014) and accounting for over 40% of the total airborne fungal spore load in Melbourne and Brisbane (Mitakakis & Guest 2001, Rutherford et al. 1997).

Many of the genera encountered in this study, such as *Cladosporium*, *Alternaria*, *Aureobasidium*, *Pestalotiopsis*, *Fusarium*, and *Colletotrichum* are ubiquitous phylloplane fungi observed regularly from a wide variety of tree species (Osono & Hirose 2009, Schulz et al. 1999). These fungi are known to be both commensal epiphytes or endophytes as well as being saprophytes; forming host epiphyte or endophyte relationships with the plant, and later becoming saprotrophic after senescence (Schulz & Boyle 2005, Vega et al. 2010). As these fungi represent primary colonisers when senescence takes place, they thus play major roles as early stage saprophytes of leaf litter (Osono 2006). In doing so, these fungi would not only contribute to the aerosolized spore load whilst behaving as endophytes, but would also proliferate post senescence, and contribute to the aerial spore load as demonstrated in this study. In contrast, a number of phyllospheric fungi rely solely on leaf exudates (Osono 2006), and thus unlikely to appear in leaf litter samples.

The diversity of fungal genera found in both leaf and air samples were greater in the higher humidity treatment across all 5 plant species tested. Many phylloplane microbes rely on the high moisture levels from leaves for growth (Vorholt 2012). After the leaf has been abscised, the availability of moisture is rapidly reduced. In the higher humidity treatment, the moisture content was evidently high enough to either maintain or facilitate growth for many of these indigenous fungi. As would be expected, xerophilic fungi were more prevalent under the lower humidity treatment; such as *Penicillium*, *Aspergillus*. These fungi create relatively light reproductive propagules that are more likely to be lifted into the air in habitats where the surface is dry (Visagie et al. 2014). Despite these genera not generally being considered as phylloplane fungi, their proliferation post senescence, as well as their presence not only from leaf samples, but also in high concentrations from air samples under lower humidity conditions is noteworthy; as these fungi may clearly make a large, if not dominant, contribution to the aeromycota of an urban environment during autumn.

Although, fungal communities in the phylloplane are known to be extremely variable in temperate regions (Vorholt 2012), some consistencies were noted; *Cladosporium* and *Alternaria* were present across all treatments, and *Trichothecium*, *Penicillium* and *Aureobasidium* occurring in the majority of samples.

Nevertheless, differences were seen across leaf samples from the various tree species, which were potentially related to variations in biotic properties (Gange et al. 2007). For example, *Platanus* leaves did not harbour *Penicillium* or *Trichothecium*, whilst all other plant species did. Although it cannot be ascertained from this study, it has been proposed that certain fungi have host preferences, having specificity for specific phyllospheric environments (Gilbert & Webb 2007, Inácio et al. 2002, Kembel & Mueller 2014). There is evidence to suggest that plant genotype can play a major role in determining the structure of phyllospheric microbial communities (Whipps 2008), as well as specific plant traits and taxonomy driving phyllospheric fungal communities (Kembel & Mueller 2014). Furthermore, the phylloplane microbial population is influenced by numerous environmental factors in addition to leaf physio-chemical properties (Whipps et al. 2008). Variations in leaf elemental concentrations, resource availability, and defensive compounds could be restricting certain fungi, while simultaneously selecting for other taxa and therefore influencing the community assembly observed on leaves (Kembel & Mueller 2014). For example, all the plant species studied here produce secondary metabolites such as flavones, flavonoids and flavonols (Alami et al. 1998, Dejon et al. 2013, Spitaler et al. 2009), which are compounds known to have antifungal properties, and which potentially alter the fungal community (Cowan 1999). Similarly, competitive and antagonistic interactions between fungal species can further alter the fungal community (Avis 2007), and may be differentially affected by different phylloplane environments on different tree species.

In the present study, some genera were found in decaying street tree leaf samples that were not present in corresponding air samples, for example: *Mycosphaerella*, *Puccinia*, *Polythrincium*, *Ustilago*, *Pestalotiopsis* and *Geotrichum*. This was considered likely to be a result of some fungal types not readily being aerosolized; some fungi being unculturable; and/or sexual fungi not forming spore bearing structures in agar culture. Further, it is anomalous that several genera of fungi were not detected in the leaf samples, but were identified from the air samples: many of the yeasts encountered, *Aspergillus* in many instances, *Chrysonillia* and *Paecilomyces*. As not all leaf matter from the chamber was assessed, there was a possibility that some taxa would evade detection. Since even very small established colonies of some species can create very large quantities of spores (Fischer & Kues 2006), these observations are unremarkable.

5 Conclusions

The phyllospheric fungi on abscised senescent autumn leaves sourced from the five most prevalent deciduous street trees of an urban environment were identified. Although differences were observed in phyllospheric fungal species presence and relative abundances across plant species, and certain fungi proliferated under different humidity levels; few differences were found in the fungal spores that were aerosolized. Very few pathogenic species were observed from the trees we sampled. It is clear from this study that phyllospheric fungi present on deciduating leaves have the potential to contribute greatly to the aeromycota of urban areas.

Acknowledgements

This study was partially funded by a research grant from the City of Sydney Council. The authors gratefully acknowledge the valuable contributions of Bill Irga, Gemma Armstrong and Russell Trenerry.

References

Agrios, G. N. (2005). Plant diseases caused by Fungi. In G. N. Agrios (Ed.), *Plant Pathology (Fifth Edition)*, (pp. 385–614). San Diego: Academic Press.

Alami, I., Mari, S., & Clérivet, A. (1998). A glycoprotein from Ceratocystis fimbriata f. sp. platani triggers phytoalexin synthesis in Platanus × acerifolia cell-suspension cultures. *Phytochemistry, 48*(5), 771–776.

Alexopoulos, C. J., Mims, C. W., & Blackwell, M. (1996). *Introductory mycology*. Brisbane: Wiley and sons.

Avis, T. J. (2007). Antifungal compounds that target fungal membranes: applications in plant disease control. *Canadian Journal of Plant Pathology, 29*(4), 323–329.

Bauer, H., Schueller, E., Weinke, G., Berger, A., Hitzenberger, R., Marr, I. L., & Puxbaum, H. (2008). Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol. Atmospheric Environment, 42(22), 5542-5549.

Beareau of Meteorology (2014). Climate data online. http://www.bom.gov.au/climate/data/ Assessed 16 September 2014.

Brown, J. K. M., & Hovmøller, M. S. (2002). Aerial Dispersal of Pathogens on the Global and Continental Scales and Its Impact on Plant Disease. *Science, 297*(5581), 537–541.

City of Sydney (2013). Urban Forest Strategy, 2013. In K. Sweeney, S. Lymbery & J. Johnson (Eds.). Sydney: The City Of Sydney.

Cowan, M. M. (1999). Plant Products as Antimicrobial Agents. *Clinical Microbiology Review, 12*(4), 564–582.

Crook, B., & Burton, N. C. (2010). Indoor moulds, Sick Building Syndrome and building related illness. *Fungal Biology Reviews, 24*(3–4), 106-113.

De Hoog, G. S., Guarro, J., Gene, J., & Figueras, M. J. (2000). *Atlas of Clinical Fungi, Second Edition*: Amer Society for Microbiology.

Dejon, L., Mohammed, H., Du, P., Jacob, C., & Speicher, A. (2013). Synthesis of chromenoindole derivatives from Robinia pseudoacacia. *MedChemComm, 4*(12), 1580–1583.

Ellis, D. H., Davis, S., Alexiou, H., Handke, R., & Bartley, R. (2007). *Descriptions of Medical Fungi* (2nd ed.). Adelaide, Australia: Women's and Children's Hospital.

Esser, H.J. (2002). The revision of *Triadica* Lour. (Euphorbiaceae), *Harvard Papers in Botany 7*(1), 17–21.

Fischer, R., & Kues, U. (2006). Asexual Sporulation in Mycelial Fungi. In *The Mycota Growth, Differentiation and Sexuality*). Berlin: Springer-Verlag.

Gange, A. C., Dey, S., Currie, A. F., & Sutton, B. C. (2007). Site- and species-specific differences in endophyte occurrence in two herbaceous plants. *Journal of Ecology, 95*(4), 614–622.

Gilbert, G. S., & Webb, C. O. (2007). Phylogenetic signal in plant pathogen–host range. *Proceedings of the National Academy of Sciences, 104*(12), 4979–4983.

Inácio, J., Pereira, P., Carvalho, M., Fonseca, Á., Amaral-Collaço, M. T., & Spencer-Martins, I. (2002). Estimation and Diversity of Phylloplane Mycobiota on Selected Plants in a Mediterranean–Type Ecosystem in Portugal. *Microbial Ecology, 44*(4), 344–353.

Irga, P. J., Torpy, F. R., & Burchett, M. D. (2014). Ecological determinants of the aeromycota in urban Sydney. In *10th International Congress of Aerobiology*. Campbelltown, Australia.

Kembel, S. W., & Mueller, R. C. (2014). Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. *Botany, 92*(4), 303–311.

Klich, M. A., & Pitt, J. I. (1988). *A Laboratory Guide to Common Aspergillus Species and their Teleomorphs*. Sydney australia: CSIRO.

Larone, D. H. (2002). *Medically Important Fungi: A Guide to Identification*, (4th ed.). Washington D.C: American Society for Microbiology Press.

Levetin, E., & Dorsey, K. (2006). Contribution of leaf surface fungi to the air spora. *Aerobiologia, 22*(1), 3–12.

Mitakakis, T., & Guest, D. (2001). A fungal spore calendar for the atmosphere of Melbourne, Australia, for the year 1993. *Aerobiologia, 17*(2), 171–176.

Mitakakis, T. Z., Clift, A., & McGee, P. A. (2001). The effect of local cropping activities and weather on the airborne concentration of allergenic Alternaria spores in rural Australia. *Grana, 40*(4-5), 230–239.

Osono, T. (2006). Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian Journal of Microbiology, 52*(8), 701–716.

Osono, T., & Hirose, D. (2009). Ecology of Endophytic Fungi Associated with Leaf Litter Decomposition. In M. Rai & P. D. Bridge (Eds.), *Applied Mycology*, (pp. 92–109). Wallingford, UK: Cambridge, CABI Publishing.

Osono, T., & Mori, A. (2004). Distribution of phyllosphere fungi within the canopy of giant dogwood. *Mycoscience, 45*(3), 161–168.

Pepeljnjak, S., & Šegvić, M. (2003). Occurrence of fungi in air and on plants in vegetation of different climatic regions in Croatia. *Aerobiologia*, *19*(1), 11–19.
Pfaller, M. A., Pappas, P. G., & Wingard, J. R. (2006). Invasive Fungal Pathogens: Current Epidemiological Trends. *Clinical Infectious Diseases*, *43*(Supplement 1), S3-S14.
Rutherford, S., Owen, J. A. K., & Simpson, R. W. (1997). Survey of airspora in Brisbane, Queensland, Australia. *Grana*, *36*(2), 114–121.
Sabariego, S., Díaz de la Guardia, C., & Alba, F. (2000). The effect of meteorological factors on the daily variation of airborne fungal spores in Granada (southern Spain). *International Journal of Biometeorology*, *44*, 1–5.
Schulz, B., & Boyle, C. (2005). The endophytic continuum. *Mycological Research*, *109*(6), 661–686.
Schulz, B., Römmert, A.-K., Dammann, U., Aust, H.-J., & Strack, D. (1999). The endophyte-host interaction: a balanced antagonism? *Mycological Research*, *103*(10), 1275–1283.
Spitaler, R., Gurschler, S., Ellmerer, E., Schubert, B., Sgarbossa, M., & Zidorn, C. (2009). Flavonoids from Celtis australis (Cannabaceae). *Biochemical Systematics and Ecology*, *37*(2), 120–121.
Tham, R., Dharmage, S. C., Taylor, P. E., Katelaris, C. H., Vicendese, D., Abramson, M. J., & Erbas, B. (2014). Outdoor fungi and child asthma health service attendances. *Pediatric Allergy and Immunology*, *25*(5), 429–449.
Torpy, F., Irga, P., Brennan, J., & Burchett, M. (2013). Do indoor plants contribute to the aeromycota in city buildings? *Aerobiologia*, *29*(3), 321–331.
Vega, F. E., Simpkins, A., Aime, M. C., Posada, F., Peterson, S. W., Rehner, S. A., Infante, F., Castillo, A., & Arnold, A. E. (2010). Fungal endophyte diversity in coffee plants from Colombia, Hawaii, Mexico and Puerto Rico. *Fungal Ecology*, *3*(3), 122–138.
Visagie, C. M., Houbraken, J., Frisvad, J. C., Hong, S. B., Klaassen, C. H. W., Perrone, G., Seifert, K. A., Varga, J., Yaguchi, T., & Samson, R. A. (2014). Identification and nomenclature of the genus Penicillium. *Studies in Mycology*, *78*(0), 343–371.
Vorholt, J. A. (2012). Microbial life in the phyllosphere. *Nat Rev Micro*, *10*(12), 828-840.
Whipps, J. M., Hand, P., Pink, D., & Bending, G. D. (2008). Phyllosphere microbiology with special reference to diversity and plant genotype. *Journal of Applied Microbiology*, *105*(6), 1744–1755.
Wickremasinghe, U. K., Heather, W. A., & Griffin, D. M. (1985). Comparative study of methods for the quantification of fungal spores at the leaf surfaces of Populus xeuramericana. *Plant and Soil*, *85*(3), 447–449.
Zak, J. C. (2002). Implications of leaf surface habitat for fungal community structure and function. In S. E. Lindow, E. I. Hecht-Poinar & V. J. Elliott (Eds.), *Phyllosphere Microbiology*, (pp. 299– 315). St Paul, USA: APS Press.