CHAPTER 7

ANALYSIS OF VERTICAL SPATIAL DISTRIBUTION OF FUNGAL COMMUNITY ON THAI DWARF FISHTAIL PALM

7.1. Introduction

Palms are a unique group within the monocotyledons (Uhl and Dransfield, 1987). Most palms are restricted to the tropics, and approximately one third of the species in the palm family (Arecaceae) are native to the Western Hemisphere (Henderson, 1995). Palms are an important component of Neotropical rainforests ecosystems where they exhibit a variety of growth forms, from acaulescent understory plants to large canopy trees (Jones, 1995). Many common products and foods are derived from palms such as various furniture from rattans, oil palm, coconut oil and its derivatives products, sago, alcoholic beverages, wine, etc., and palms are also widely used in landscaping for their exotic appearance, making them one of the most economically important plants (Johnson, 1992). In addition to their beauty, palms perform a number of environmental services (Johnson, 1992). For examples, decomposing palm fronds create much-needed organic soils and provide shelter for snakes, lizards and insects. The trees themselves provide homes for a variety of plants, animals, fungi and microorganisms. In an environment where food is extremely hard to come by, the tasty and edible fruits of the palm trees provide a necessary food source for many species including birds and other animals. In exchange for providing nourishment, the birds and other animals freely disperse the seeds of palms along with

a convenient dose of bio-fertilizer. This mutual arrangement ensures the survival of both animals and palms (Johnson, 1992).

Microhabitat is defined as a very small, specialized habitat within a habitat that possesses unique properties where new variations of life can exist and thrive due to the unique conditions that the microhabitat offers, such as a clump of grass or a space between rocks. Different parts of palm fronds such as pinna, petiole and rachis could also be grouped as microhabitat including also hanging and fallen fronds. Fungi are spatially structured in the environment in response to a number of biotic and abiotic features. In relation to plant as their habitat, in smallest scale, fungi are structured in response to vegetation pattern such as size, spacing, root distribution and the distribution of vegetative resources such as exudates, leaf litter, stem flow and throughfall (Morris and Robertson, 2005). However, at the microscale, current methodology to understanding fungal ecology is limited by techniques and approaches currently available.

Most of the fungal ecological studies, particularly on palms, have employed fungal fruiting bodies appearance and fungal spores production analysis technique (Fröhlich and Hyde, 2000; Yanna, 2001, Yanna *et al.*, 2001; Taylor and Hyde, 2003). From those studies, it was clear that biogeography, climates (microclimate and macroclimate), host proximity (e.g. occurring in the same location) and plant parts (e.g. leaves, rachis, petioles) are key factors affecting the fungal community occurs on various plants. In fact, different ecosystem also possesses very variable microclimates and other specific environmental factors. Although several ecological aspects of palmicolous fungi have been studied and reported (Fröhlich and Hyde, 2000; Yanna, 2001, Yanna *et al.*, 2001; Taylor and Hyde, 2003; Pinnoi *et al.*, 2004; Pinruan *et al.*, 2007), however, little is known about spatial distributions of fungal communities on palms such as the fungal community on decaying fronds before and after falling into the soil floors/grounds (vertical distribution). In order to begin and develop a more understanding of spatial patterns of fungal community structure, studies describing spatial distribution of fungal community are necessary.

This chapter was carried out in order to test the hypothesis of that the fungal community patterns of palms is affected by space/spatial distribution (Morris and Robertson, 2005) in particular vertical spatial distribution (Robinson *et al.*, 2009), in this case, hanging (not touching the grounds) and fallen fronds (touching the grounds). Thus, in this chapter, pattern and variation between fungal community on hanging and fallen fronds are presented including their diversity and similarity.

7.2. Material and methods

Collection and Experimental Design

Standing fronds were sampled as follows: on early of rainy season, two fronds were cut at the bottom level and divided into 2 standardized, one frond was tied on the palms tree (hanging fronds) and another one was placed on the ground/forest floor under the palm tree (fallen fronds). Each frond consists of primary rachis, secondary rachis and pinna. Collections were carried out after 12 months. The examination was carried out in 10 replicates. All field samples were put in 11.5" x 16.5" resealable bags. Collecting bags were sealed and labeled as follows: *Name of the palm, Collecting site, Collector/s, Date.*

Examination of Material

On returning to the laboratory, the materials were immediately examined periodically over the next month. The decaying and senescent materials were examined for saprophytic Ascomycetes, Coelomycetes, Hyphomycetes and Basidiomycetes. The materials were examined using an Olympus SZ H10 dissecting microscope to determine the presence of the fungal fruiting structures and Olympus BX51 to determine microscopic structures. Once fully examined, the pieces of materials were air dried and placed in a resealable envelope with the following information: *Herbarium number, Host name, Collection site, Collector, Date.* Dried herbaria of interesting specimens were deposited at CMU Herbarium (CMU), Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

Data Analysis

A total number of species and the number of fungi per sample were recorded and calculated. Species-area curves were plotted for each collection to examine the sample size (Begon *et al.*, 1992). Dominance diversity curves were plotted as a reflection of the relative abundance of species in each microhabitat sample (Kent and Coker, 1992). Percentages abundance, recurrence and frequency of occurrence of each species were employed in order to compare the dominance of fungi among different collections (Cai *et al.*, 2006) as follow:



The taxa with a percentage of occurrences higher than or equal to 10% are considered to be common species.

Margalef's index was employed to analyze the species richness of fungal community at each microhabitat designed. Shannon-Weiner diversity indexes (H'), which incorporate species richness and species evenness (Begon *et al.*, 1992), was applied to evaluate the diversities of fungal communities. Species richness refers to the number of species in a community and species evenness refers to the contribution (relative abundance or equability).

Shannon diversity index $\mathbf{H} = -\sum \mathbf{p}_i \cdot \mathbf{ln} \mathbf{p}_i$ (\mathbf{p}_i : proportion of species \mathbf{i}^{th})

(The higher of the Shannon diversity index is, the more diverse is the community). Species evenness $\mathbf{E} = \mathbf{H}/\mathbf{lnS}$ (S: total species number)

(Shannon evenness accounts the equability of species present (E) (Gotelli and Colwell, 2001). Shannon evenness ranges from 0 to 1. If one community with Shannon

evenness index equal to 1, it means that distribution of every species in the community is equal).

Similarities among the fungal communities from different part of fronds was calculated by using Sørensen's index of similarity (S') with values between 0 (no similarity) and 1 (absolute similarity) (Magurran, 1988). A *t*-test was also performed to compare the Shannon-Weiner indices between different fungal communities (Hutcheson, 1970).

Hierarchical Cluster Analysis (HCA) was performed to classify species recorded on the palms fronds. Species with total recorded (> 10) were selected as cases in the analysis. Ward method was used as a clustering method, Squared Euclidean Distance was selected as an interval measurement and Z-scores was used to standardize the transform value of variable group.

In order to constructs a configuration/map of the fungal community on specimens examined, Multi Dimensional Scaling (MDS) ALSCAL analysis using Euclidian model distance was performed based on dissimilarity matrices and downweighting of rare taxa (with frequency of occurrence < 10%). The relationship between assemblage of the fungal community (with frequency of occurrence > 10%) and different type of palm tissues was also analysed using a simple correspondence analysis. Two dimensional plots were generated from the MDS and Correspondence Analyses (CA).

All analyses were performed using SPSS 16.0 software (Anonymous, 2007) and XLSTAT-Pro version 7.5 (Anonymous, 2004).

7.3. Results

Species area curve was plotted to indicate the increasing number of fungi with the number of samples examined (fig. 7.1). The asymptotes were reached at around 27-33 samples (fig. 7.1). Therefore, the samples size (100 samples for each experimental treatment) used in this investigation can provide a reasonable estimates of the fungal community in this particular ecological niche.



A total 200 specimens of palm fronds (100 from the hanging fronds, 100 from the fallen fronds) were examined for the presence of fungal fruiting bodies, yielding 52 microfungi taxa including 21 Ascomycetes (representing 41% of all taxa), 9 Coelomycetes (17%) and 22 Hyphomycetes (42%) (fig. 7.2). Based on total number of records/abundance dataset, 816 total number of records were recorded from all taxa consist of 33% Ascomycetes (representing 272 abundances), 26% Coelomycetes (211 abundances) and 41% Hyphomycetes (333 abundances) (fig. 7.2).



Diaporthe palmarum (Σ records = 68, FO = 34%), Dictyochaeta wallichianensis (Σ records = 67, FO = 33.5%) and Chaetospermum chaetosporum (Σ records = 63, FO = 31.5%) appeared as most dominant species from total specimen observed (appendices 3, 5; fig. 7.3). A detail list of taxa with their Σ records, total frequency of occurrence and % abundances are presented in appendix 7. A more detail information of Σ records, total frequency of occurrence, % recurrence and % abundances of all taxa at every site and experimental treatment (hanging-fallen frond) is also given in appendix 8. The detail frequency of occurrence of all taxa at each designed microhabitat is also presented in appendix 9.



A proportion of fungal community based on qualitative (Σ taxa) and quantitative (Σ records/abundance) dataset when all taxa were grouped according to the experimental design (hanging and fallen fronds) after 12 months decomposition in nature is presented in figures 7.4 and 7.5. Taxa belong to Ascomycetes were frequently found on hanging fronds during this study with 22 taxa recorded (Σ records = 171) (figs. 7.4, 7.5). On fallen fronds, hyphomycetous taxa appeared as the most common group with 20 taxa recorded (Σ records = 207) followed by Ascomycetes (11 taxa, Σ records = 101) and Coelomycetes (10 taxa, Σ records = 83) (figs. 7.4, 7.5). When the fungal community was categorized based on the tissue types, taxa belong to Hyphomycetes were very dominant on primary and secondary rachis, qualitative and quantitatively, followed by Ascomycetes and Coelomycetes (figs. 7.6 and 7.7).





Fig. 7.5 Quantitative distribution of all taxa recorded on *Wallichia siamensis* based on experimental design. All taxa grouped based on their pseudo-systematic position.





When the dataset of tissue types and experimental design were combined, the following four microhabitats were yield, namely, hanging-primary rachis (HPR), hanging-secondary rachis (HSR), fallen-primary rachis (FPR) and fallen-secondary rachis (FSR). Based on these microhabitats classification, qualitatively (Σ species), hyphomycetous taxa appeared as a dominant group on HPR, FPR and FSR with 15, 12 and 8 total number of taxa recorded, respectively (fig. 7.8). On the other hand, ascomycetous taxa were dominant on HSR with 10 total number of taxa recorded (fig. 7.8). Based on quantitative data (Σ records/abundance), ascomycetous taxa appeared as a dominant group on HPR and FSR with 101 and 70 total numbers of records, respectively (fig. 7.9). Taxa belong to Hyphomycetes were very dominant on FPR and FSR with 112 and 95 total numbers of records, respectively (fig. 7.9).



In the present study, average number of records per sample was also measured (figs. 7.10, 7.11 and 7.12). On hanging fronds, taxa belong to Ascomycetes apparently showed highest average number of records per sample followed by Coelomycetes and Hyphomycetes, with 1.7, 1.3 and 1.3 average number of records/sample, respectively (fig. 7.10). Even though taxa belong to Hyphomycetes showed the lowest average number of records per sample on hanging fronds, however, this group showed the highest average number of records per sample on fallen fronds with 2.1 records/sample (fig. 7.10), as well as on primary rachis (1.9 records/sample) and secondary rachis (1.4 records/sample) when tissue types dataset was analysed (fig. 7.11).



Fig. 7.10 Average number of record per sample all taxa recorded on *Wallichia siamensis* based on experimental design. All taxa grouped based on their pseudo-systematic position.



Based on the combination of experimental design-tissue types (4 microhabitats performed), taxa belong to Ascomycetes showed the highest average number of fungi records per sample on HPR and HSR with 2 and 1.4 records/ sample, respectively (fig. 7.12). Hyphomycetous taxa showed the highest number of fungi records per sample on FPR and FSR, with 2.2 and 1.9 records/sample, respectively (fig. 7.12).



Fig. 7.12 Average number of record per sample all taxa recorded on *Wallichia* siamensis based on combination of experimental design-tissue types. All taxa grouped based on their pseudo-systematic position.

Species abundance curve or dominance-diversity curve was plotted and shown in figure 7.13. The plot showed that there were about four species of fungi with more than 15 abundances appeared on all microhabitats (HPR, HSR, FPR, FSR), and only three species of fungi appeared with more than 25 abundances. Each species represented HPR, HSR and FSR, respectively (fig. 7.13). This data indicated only a few taxa were dominant on *W. siamensis* after one year decomposition.



After one year decomposition in natural environment, several common palmicolous fungi genera previously reported by Fröhlich and Hyde (2000) and Taylor and Hyde (2003), such as *Anthostomella*, *Arecomyces*, *Lophiostoma*, *Myelosperma*, *Ophioceras*, *Oxydothis*, *Pemphidium* and *Rachidicola*, were also encountered in this study (appendix 7). Overall, the most common taxa with frequency of occurrence more than 30% were *Diaporthe palmarum*, *Dictyochaeta wallichianensis* and *Chaetospermum chaetosporum* with frequency of occurrences (FO) of 34%, 33.5% and 31.5%, respectively (appendix 8). It was also found that Chaetospermum chaetosporum, Diaporthe palmarum and Phomopsis caryotae*urentis* appeared as dominant taxa on hanging fronds of *W. siamensis* with frequency of occurrences of 47%, 43% and 36%, respectively (appendix 8). On the other hand, Dictyochaeta wallichianensis (FO = 57%), Beltraniella portoricensis (FO = 47%) and Beltrania rhombica (FO = 29%) appeared as common species on fallen fronds (appendix 8). Based on the frequency of occurrence analysis resulted from tissue Diaporthe palmarum, types dataset, Dictyochaeta wallichianensis and Chaetospermum chaetosporum appeared as dominant species on primary rachis with frequency of occurrences of 45%, 35% and 32%, respectively (appendix 4). In addition, Phomopsis caryotae-urentis (FO = 38%), Dictyochaeta wallichianensis (FO = 32%) and Chaetospermum chaetosporum (FO = 31%) were dominant in fungal community on secondary rachis (appendix 8).

Two dimensional graphic generated from MDS analysis illustrated a grouping taxa based on their frequency of occurrence values from total specimen examined (fig. 7.14). A vertical axis of the diagram (Dim1) separated the most frequent taxa in this study into two distinct groups (fig. 7.14). The right large cluster consists of taxa with FO occurrence > 20% and another large cluster in the left side consists of taxa with FO < 20% (fig. 7.14, appendix 8). The separation of these two distinct fungi groups was also supported by hierarchical cluster analysis (fig. 7.15). The graphic generated from the hierarchical cluster analysis apparently classified the taxa into two major clusters. The first cluster (below) consists of taxa with frequency of occurrence less than 20% and the second cluster (upper) consists of taxa with FO over 20% (figure 7.15).



Fig. 7.14 Two dimensional of Multi Dimensional Scaling (MDS) ordination of all taxa with frequency of occurrence > 10% based on total abundances and Euclidean distance model. Taxa showed by acronyms.

— : Small cluster grouping; ---- : Larger cluster grouping.

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By performing correspondence analysis (CA) in analyzing the relationship between taxa and tissue types, it was also clear that several taxa were well separated in concordance with tissue types and experimental design (fig. 7.16). Axis 1 (F1) separated taxa between hanging and fallen fronds (fig. 7.16). On the other hand, axis 2 (F2), separated taxa between primary and secondary rachis (fig. 7.16). When dataset of tissue types and experimental design were combined (4 microhabitats performed), several taxa indicated a close relationship with the designed microhabitats. On HSR, taxa such as *Phomopsis caryotae-urentis*, *Lasiodiplodia theobromae*, *Chaetospermum chaetosporum* and *Montagnula* sp., appeared as common taxa (fig. 7.16), as well as *Diaporthe palmarum* on HPR (fig. 7.16). *Myelosperma tumidum* appeared as a common species on FSR, and several hyphomycetous taxa such as *Beltraniella portoricensis*, *Beltrania rhombica*, *Dictyochaeta wallichianensis* and *Helicomyces liliputeus*, appeared as common taxa on FPR (fig. 7.16). This data was also clearly illustrated using contingency table (fig. 7.17).





Box plots illustration illustrated the distribution of dominant species on different tissue types and location/space (experimental design) after one year decomposition (fig. 7.18). Coelomycetous taxa, *Chaetospermum chaetosporum* and *Phomopsis caryotae-urentis* occurred more frequently on hanging fronds than on fallen fronds based on their distribution on different location/vertical spatial distribution (fig. 7.18). Both taxa were also more frequently found on secondary rachis than primary rachis (fig. 7.18). On the other hand, *Diaporthe palmarum* indicated an equal distribution on all designed categories (tissue types and experimental design). However, this taxon showed higher abundance on hanging fronds than fallen fronds, as well as on primary rachis than secondary rachis (fig. 7.18).



siamensis after one year decomposition based on experimental design and tissue types. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values, and [°] indicates outliers.

According to the box plots diagram generated from Shannon-Weiner diversity, Margalef's species richness and abundance datasets analyses, it was apparent that fungal diversity on hanging frond (H' = 3.2; Margalef's index = 2.9) was higher than fallen frond (H' = 3.1; Margalef's index = 2.5) (figs. 7.19, 7.20). A distribution of fungal community on hanging frond were less even than fallen frond due to one or two sites where the specimens collected possess a very high diversity than other sites. These results were also confirmed by the presence of outliers on hanging fronds samples. In contrast to diversity and species richness indices, fungal community abundance on hanging fronds was lower than fallen fronds (fig. 7.21). The abundance of fungal community of hanging fronds at each site was also not evenly distributed due to contains high and extremely high abundance at two sites where the specimens collected (showed by outliers).

When the dataset based on experimental treatment was analysed, diversity level of fungal community on primary rachis (H' = 3.3; Margalef's index = 3.2) was higher than secondary rachis (H' = 3.1; Margalef's index = 2.6) (figs. 7.19, 7.20), however, the fungal community on primary rachis (E' = 0.9) was less dispersed than secondary rachis (E' = 0.9) which indicated low evenness fungal diversity on this tissue type (appendix 9).



Fig. 7.20 Box plots showing Margalef's index species richness of fungal community of *Wallichia siamensis* based on combination of experimental design and tissue types. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values, and "indicates outliers.



Fig. 7.21 Box plots showing abundances of fungal community of *Wallichia siamensis* based on experimental design and tissue types. The lower and upper boundaries of each box enclose 25-75% of the data. The line within the box shows the median values, the bar lines above and below the boxes indicate minimum and maximum values, and [°] indicates outliers.

7.4. Discussion

Are palmicolous fungi space preference?

The present study confirmed that overall fungal community on *W. siamensis* possessed a broad taxonomy distribution due to consisting of three major pseudosystematic taxa, namely, Ascomycetes, Coelomycetes and Hyphomycetes. On this palm, the member of Hyphomycetes occurred at very high frequencies, followed by Ascomycetes and Coelomycetes (fig. 7.2). This composition was similar to the fungal community composition on hanging fronds where the ascomycetous taxa were very dominant with 46% of total species recorded (figs. 7.22; 7.23). These results indicated the effects of space, in particular spatial vertical distribution (designed in this study), to the fungal community structures occur on palm tissues. Decaying fronds not touching the grounds (hanging fronds) and touching the grounds (fallen fronds) were hypothesized in the present study as specialized microhabitats within a habitat that possess unique properties where new variations of life can exist and thrive due to their unique conditions offers, such as temperatures, humidity, sunlight intensity, vegetation shading and other environmental factors.





The spatial vertical distribution preference of fungal community on palm was also supported by the Sørensen's index of similarity (S) analysis. It was apparent that higher similarity (S') was encountered between the fungal communities on tissue types (primary-secondary rachis = 0.6) than vertical spatial distribution (hanging-fallen fronds = 0.5) (fig. 7.24). Since the fungal communities on hanging and fallen fronds were less similar than based on tissue types (primary-secondary rachis), therefore, it was clear that the fungal communities based on vertical spatial distribution were more diverse. It strongly supported the importance of vertical spatial distribution to the fungal community structure and composition occurs on palm tissues. Therefore, this result suggested that space (vertical distribution) is also invaluable parameter in the study of fungal community on plants tissue.



Sorensen index analysis.

This factor even greater affecting the fungal community structure and composition than tissue types as previously suggested by Yanna *et al.* (2001) and Taylor and Hyde (2003). Unfortunately, the two tail of *t*-test analysis accepted the hypothesis that fungal community on primary-secondary rachis and hanging-fallen fronds were equal (95% confidence) (table 7.1). This fact, probably due to an inadequate number of samples examined, and therefore, larger dataset and sample are probably necessary in order to statistically support the present finding.



The pattern of higher fungal community on the hanging fronds than fallen fronds was probably related to the heterogeneity in micro-environmental factors between the two microhabitats such as sun exposure, humidity and water availability. A similar result was also reported by Andrews *et al.* (1980) who noted the higher microbial populations within a plant canopy on leaves than the one closing to the ground. However, the reason for this pattern is still not clear. Traditionally, the microbiogeography of an organism or class of organisms in a habitat is differentiated on the basis of physical environment (such as temperature and moisture) and availability of food (Andrews and Harris, 2000). For examples, on the rhizoplane, it probably relates to variation in nutrients (Andrews and Harris, 2000), and on the phylloplane, it relates to accumulation of cells in depressions as the leaves dry, sun exposure and leakage of nutrients (Canny, 1990); or to sheltering of cells from environmental stresses (Canny, 1990). In general, Deacon (2006) also insisted a fluctuation of several environmental factors such as exposure to UV irradiation, temperature, moisture and nutrient levels also significantly affects to the composition of fungal community.

Probably, the most obvious explanation regarding the difference between microfungi communities on hanging and fallen fronds was illustrated by Andrews and Harris (2000). The major plant surface can be considered as micro-biogeographic zones (fig. 7.25) as a function of increasing exposure to air and sun (vertical axis) and increasing availability of organic nutrients from oligotrophy (sparse organic nutrients) to dominant or transient copiotrophy (abundant organic nutrients) (horizontal axis).



Fig. 7.25 The major biogeographic zones for microbial colonization of plant surfaces as a function of exposure and nutrient availability (from Andrews and Harris, 2000).

Andrews and Harris (2000) insisted that different rhizoplane (representative of below-ground epiphytic habitats) and phylloplane surfaces (representative of above-ground epiphytic habitats) vary in terms of:

(a) Extent of exposure to the sun, wind and rain, and related moisture and aeration conditions of the habitat;

(b) Forms and amounts of plant photosynthate available as a food resource or as a signaling mechanism between microbial and plant cells;

(c) Topography;

(d) Longevity (length or duration of life).

The present study also illustrated that a changing in fungal community structures during decomposition processes was also related with the changing in substrates structures. Even though all specimens of hanging and fallen fronds were treated equally by incubating in natural environment for one year, however, the decomposition rates of hanging and fallen fronds were distinctly different. This was not only because of the fungal community sequential changing, but also other factors such as exposure of the sun, moisture, water availability and invertebrate fauna as suggested. The dominance of ascomycetous taxa only on hanging fronds clearly showed that a decomposition rate of hanging fronds was slower than fallen fronds. By analyzing the time for fungal communities to reach the peak of species diversity, it is possible that the fungal community on hanging fronds was still at peak of species diversity (mature community) due to the dominance of ascomycetous taxa after one year decomposition. On the other hand, the fungal community on fallen fronds was already over the peak of species diversity (impoverished community) due to the dominance of a few species, in particular anamorphic fungi. This result was also supported by the fact that pinna/palms leaflet of fallen fronds were completely decomposed after one year incubation, therefore, the datasets from pinna were not available to be analyzed.



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