The ecology of *Aspergillus fumigatus* and implications for wildlife conservation in modified environments
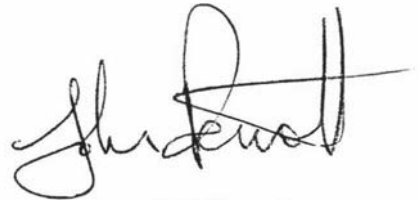
John K. Perrott

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Declaration of Originality

This thesis represents the original work of the author, except where otherwise acknowledged. It has not been submitted previously for a degree at any university

John Keith Perrott

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Abstract

This thesis outlines my research since 1998 into inter-trophic interactions between pathogenic fungi, insects, birds, and the environment. Chapter One is a stand-alone investigation into fungal diseases associated with native cicadas on Mokoia Island (Lake Rotorua) and the Eastwoodhill Arboretum (Gisborne). Chapters Two through Four are specifically concerned with the natural occurrence of the pathogenic fungus *Aspergillus fumigatus* and its connection to hihi (or stitchbird) on Little Barrier Island (northern Hauraki Gulf), Mokoia Island, Tiritiri Matangi Island (Hauraki Gulf), and the Mt Bruce Wildlife Centre (near Masterton).

The hihi (*Notiomystis cincta*) is a small (i.e., starling sized) cavity-nesting honeyeater endemic to New Zealand. Following European colonisation, hihi became extinct everywhere except on Little Barrier Island, making the future of this species very uncertain. In response to this situation there have been several translocations of hihi to Hen, Cuvier, Kapiti, Mokoia and Tiritiri Matangi Islands from 1980 to 1996. While hihi on Tiritiri Matangi seem to be progressing well with intensive management, all previous translocations to other islands have failed to establish self-sustaining populations. The main hypotheses given to explain these failures are insufficient year-round supply of nectar and fruit, and lack of suitable nesting cavities.

Hihi were translocated to Mokoia Island in September 1994 with nest boxes provided. However, like past translocations, hihi have continued to decline on the island with high annual mortality rates above 50%. It is not clear what factors are contributing to this high mortality rate. My MSc studies carried out on Mokoia Island during 1994-1997 demonstrated that hihi had a good year-round supply of food, and were not dying as a result of starvation. Subsequent post-mortem examinations by Professor Alley of sick and dead hihi from Mokoia from 1995-1997 has revealed that the fungal disease aspergillosis, caused by *Aspergillus fumigatus*, is the most common cause of death among those birds. In addition, captive hihi at the Mt Bruce Wildlife Centre also suffer high rates of aspergillosis, and there is presently concern regarding the susceptibility of hihi to this fungal disease. This study suggests also that past hihi translocations have failed due, in part, to birds being exposed to elevated levels of disease-causing microorganisms.

Aspergillus fumigatus is an ubiquitous fungus, and common aspergillosis-causing pathogen in birds and mammals. Infection takes place following the aspiration of fungal spores, and is primarily a respiratory disease. *Aspergillus* infections are usually considered to be opportunist following other primary infections, immunosuppression or stressful environmental conditions. Small numbers of spores can usually be tolerated by the immune system, but large numbers can cause disease and death. At present, prevention is the only effective method of controlling aspergillosis in wild bird populations.

This project is concerned with studying the ecology of *A. fumigatus*, and identifying the most likely source/s of infection in hihi. Therefore, this project is directed towards identifying the most likely source/s of *A. fumigatus* rather than the most likely causes of aspergillosis in hihi. In doing so, the primary aim of this project is to investigate

whether previous hihi translocations from Little Barrier Island to young growth forests and forest edge habitats have been exposing birds to elevated levels of *A. fumigatus*, and therefore, whether *A. fumigatus* preventative management should be considered when planning future hihi translocations.

This project aimed to, A) determine whether habitat disturbance encourages the establishment of *A. fumigatus* in the environment B) measure and compare *A. fumigatus* densities from early growth regenerating forests (i.e., Mokoia and Tiritiri Matangi Islands), a pristine mature forest (i.e., Little Barrier Island), and two mainland forest locations (i.e., Mt Bruce Wildlife Centre, and the Massey University Campus) C) measure and compare *A. fumigatus* densities in forest edge habitats and inner forest locations. D) investigate whether hihi nest boxes on Mokoia Island and natural tree cavity-nests on Little Barrier Island are promoting the growth of *A. fumigatus* and, E) investigate various types of forest disturbance events that could account for the differences in *A. fumigatus* densities found between study sites.

Results from Chapter One describe a unique and previously unreported cicada disease caused by a *Conidiobolus* fungus on Mokoia Island. Results from Chapters Two and Three report elevated levels of *A. fumigatus* on Mokoia Island and at the Mt Bruce Wildlife Center. Low levels of *A. fumigatus* were recorded on Little Barrier Island, and moderate levels recorded for Tiritiri Matangi Island. Results indicate that forest disturbance promotes the abundance of *A. fumigatus* in the environment, and that forest edge habitats have significantly higher levels of *A. fumigatus* compared to inner forest locations. This suggests that forest disturbance alters natural disease dynamics, and increases bird`s exposure to opportunistic disease-causing agents such as *A. fumigatus*. These results, in part, illustrate the functional significance of old growth forests in keeping weedy pest species like *A. fumigatus* in check. Results from Chapter Four report that hihi are exposed to elevated levels of airborne *A. fumigatus* spores while in the nest box. Additionally, results indicate that hihi re-using old nest sites would further suffer increased exposure to *A. fumigatus* spores.

Because hihi are a vulnerable species, and seem to be particularly susceptible to aspergillosis, the conservation value of this work is high. This study was made possible by grants from the J.S.Watson Conservation Trust, Massey University Graduate Research Fund, and the World Wide Fund for Nature, and furthers our understanding of mortality factors affecting free-living birds, and provides new information on the ecology of this common wildlife disease. The broader implications of this work for managing wildlife, particularly birds in New Zealand forests are also explored. The relationship between clear-felling forest regrowth, and the incidence of aspergillosis may be the key to understanding the fate of bird populations re-introduced into forests, and determine the viability of "mainland islands".

Acknowledgements

Chapter 1

General introduction

Disease emergence and reemergence among human populations have been hallmarks of the latter part of the twentieth century (Centre for Disease Control 1994; Levins *et al.* 1994, DaSilva and Laccarino 1999; Gratz 1999). In addition, infectious and non-infectious wildlife diseases are being recognised by conservation biologists as an increasing challenge to the conservation of threatened species (Friend *et al.* 2001; Deem *et al.* 2001). The role of wildlife disease as a factor limiting the survival of wildlife in 'natural' ecosystems has been linked to anthropogenic changes and habitat modification at both national and global scales (Lande 1993; Friend 1995; Patz *et al.* 2000; Deem *et al.* 2001). These changes include human population growth, habitat fragmentation and degradation, the isolation of populations of species, and an increased proximity of humans, and their domestic animals, to wildlife. Additionally, some conservation projects have caused more harm than good by unwittingly introducing diseases to wildlife populations, whereas others have failed to meet their objectives because they did not take disease factors into consideration (Deem *et al.* 2001). This suggests that contemporary conservation biologists need to consider more ways to integrate wildlife health components into the design and implementation of their wildlife studies to ensure the effectiveness of their efforts.

If one reads recent editorials and reviews in scientific journals, it is easy to come away with an impression that disease and health issues have only just been discovered as important factors in the conservation of wildlife (Meffe 1999; Daszak *et al.* 2000). In fact, for over 60 years wildlife disease has been recognised as an important issue in the conservation of threatened species (i.e., Leopold 1933). What is new, however, is the increasing attention the subject is receiving from the wider conservation community (Deem *et al.* 2001). This might suggest that we are poised to move beyond the present single-species approach to wildlife disease management, and start identifying environmental factors that can be used in disease prevention management. To obtain this goal we are going to have to start viewing wildlife disease as an outcome, rather than a cause. This will require greater understanding on the ecology of disease-causing agents in order to identify the most vulnerable linkages between affected species, the disease agent/s, and environmental factors resulting in disease. This understanding is fundamental to the development of effective disease prevention and control strategies.

European colonisation of New Zealand has effected an unprecedented redistribution of our native fauna and flora. Anthropogenic change has carved a highly modified fragmented landscape characterised by young growth and forest edge habitats. Because of this, we are increasingly forced to manage many threatened species in predator-free regenerating forests, such as hihi (or stitchbird, *Notiomystis cincta*) on Mokoia Island (Lake Rotorua). Some of our native avifauna, such as the saddleback (*Philesturnus carunculatus*) appear to thrive in Mokoia type habitats, whereas others like hihi suffer high mortality rates. Both are tree cavity-nesting species, will use nest boxes when provided, and both were once considered predisposed to mature forest habitats. The decline of hihi on predator free islands has prompted new concerns regarding the long-term management of hihi in modified environments, and interest in the relative role mammal predators may have played in their extinction on the mainland. The recent success with hihi on Tiritiri Matangi Island has shown that hihi survival in regenerating forests can be enhanced with intensive management. Because it is not viable to manage hihi indefinitely, the long-term survival of hihi probably depends on the establishment of self-sustained populations in modified habitats. The progress with hihi on Tiritiri Matangi is still scant protection if the only self-sustained hihi population goes into decline on Little Barrier Island (LBI). This has focused attention on identifying factors that can be used to gauge habitat suitability for future hihi translocations. This thesis reports work that was designed to address questions related to why hihi on Mokoia Island seem susceptible to the fungal disease aspergillosis, and whether habitat type has an influence on the abundance of the primary disease-causing fungus, e.g., *Aspergillus fumigatus*.

This thesis was initiated with a comparative study of the densities and distributions of native cicadas on Mokoia Island and at Eastwoodhill Arboretum (Gisborne). Most cicada species are forest edge insects and occur in highest densities in young growth vegetation. This is because adult cicadas prefer to lay their eggs in small twigs two to three years old, and young growth and forest edge vegetation tend to have elevated growth rates compared to old growth vegetation (White and Pickett 1985). In fact, cicadas will commonly emerge from old growth habitats and seek out adjacent young growth vegetation, often younger than the cicada itself (White 1980). The cicada component to this study led to finding fungal infected cicadas discarded inside and outside hihi nest boxes on Mokoia Island. Prior to this discovery, another study concerned with disease related mortalities in the hihi population on Mokoia identified the fungal disease aspergillosis as the most common cause of death among birds sampled (Alley et al. 1999). Hihi feed their chicks on invertebrates such as cicadas, and the discovery instigated an investigation into fungi associated with cicadas on Mokoia Island and at Eastwoodhill Arboretum. Consequently, *A. fumigatus* was isolated from all cicada remains found discarded in nest boxes on the island. This situation could be elevating hihi exposure to *A.*

fumigatus while in the nest box, and prompted a change in direction for the project to concentrate more closely on the ecology of *A. fumigatus* in modified environments. The cicada component to this thesis established that fungal disease was common among Mokoia cicadas, and uncommon among cicadas at Eastwoodhill Arboretum (EWH). Because of the relative scarcity of cicadas and fungal disease at EWH and the fact that hihi are not present at EWH, I decided against a second year at the arboretum in order to concentrate on Mokoia and other islands that have resident hihi. Nevertheless, the EWH data is still included in this thesis for comparison, and represents new information collected from an understudied group of insects in New Zealand.

Small cicadas are commonly observed being hawked by hihi, which they promptly de-wing and often consume whole. However, large cicadas such as *Amphipsalta zealandica* are not consumed whole by hihi, instead they tend to egest the abdomen only and discard the carapace and wings. At times these cicada leftovers are discarded within the nest box, and become incorporated into the detritus layer on the nest box floor. An active nest box is an invariably dark, warm, high humidity environment, and seems ideally suited to the growth and proliferation of most saprophytic fungi. Hihi provision their nest boxes with an assortment of natural materials composed mostly of dry twigs, fern hairs, lichens, leaves, feathers, and grasses. These materials, as well as the foodstuffs brought into the nest box will be covered in fungal spores derived from their environment. In addition, airborne spores will readily enter the nest box via the entrance hole. Therefore, the microflora of the nest box will probably reflect the microflora of the external habitat.

The cicadas and the fungal disease-causing agent/s themselves are both components of the hihi habitat on Mokoia, and probably typical to most forest edge habitats in New Zealand (this study). This suggests that hihi residing in young growth and/or forest edge habitats may suffer elevated exposure to *A. fumigatus*, and the materials they bring into the nest box may compound this. However, to prove that hihi have lower survival rates in forest edge habitats compared to old growth forests requires more information on the survival and reproductive rates of hihi on Little Barrier Island (LBI). This is because the only viable hihi population surviving in mature forest is found on LBI. The reproductive outputs of hihi on Mokoia seem comparable to LBI (e.g., Angehr 1984, 1985; Rasch 1985a,b) and other recipient islands (e.g., Castro 1995; Armstrong et al. 1996). Therefore, there is little evidence that Mokoia hihi suffer reduced fecundity to a degree sufficient to account for their decline on the island. However, there is no hihi survival data available from LBI to compare to hihi survival data collected from other islands. While the picture is incomplete, tentative evidence suggests that the decline of hihi on recipient islands has been caused by low survival rates, and not by low reproductive rates (Armstrong et al. 1996).

This thesis touches on a range of topics and different organisms, and has evolved in ways I did not envisage. However, there are two dominant themes underlying all chapters. First, that there is need for a greater understanding of the role of fungi in ecological systems, and this understanding is needed for more effective management of both pest and threatened species. Second, that understanding the effects of fungal disease on wildlife requires a greater understanding of the ecological factors affecting the fungi themselves and less focus on disease transmission. In this chapter I set the scene by giving a basic outline of 1) the role of fungi in forest ecosystems, 2) diseases and disease-causing agents, 3) *Aspergillus* and aspergillosis, 4) the potential role of aspergillosis in the decline of hihi, and 5) outline of other chapters.

1) Role of fungi in forest ecosystems

Ultimately species effects on nutrient cycling in terrestrial systems are the direct result of microbial activities in the soil. Many microorganisms play an essential role in decomposing plant and animal remains and in converting nitrogen from the air into forms that plants can use (Atlas and Bartha 1998). Differences in the chemical composition of the soil along with environmental factors, such as moisture and temperature, have direct effects on microbial community structure. At the same time, differences in microbial functional groups can have direct effects on plant community structure. Microorganisms occupy a greater range of ecological niches compared to plants and animals (Price 1988), and can occur in all niches where life is thermodynamically possible. The diversity, composition, and structure of microbial communities in modified habitats seems poorly understood, yet it is probably through externally generated disturbance events and elementary microbial processes that many plant and animal functional groups are detectable at the ecosystem level (Zak *et al.* 1994; Schimel 1995).

The effect of temperature on the stability and survival of fungal spores and conidia often depends on soil humidity. At the soil surface, moisture is usually in equilibrium with the atmosphere at less than 95% RH (Atlas and Bartha 1998). In these types of habitats growth of most fungal species are restricted or completely inhibited. Dry poultry house litter low in moisture tends to contain fungal species which are characteristic of low moisture environments, such as members of the *Aspergillus* group. The presence of these species are significant because they can initiate growth at high temperature/low moisture levels and then gradually release water via their metabolic activities. In doing so, they facilitate the growth of other moulds such as *Mucor* and *Penicillium* (Dennis and Gee, 1973).

Seasonal fluctuations of fungi have been recorded by several authors (e.g., Brierley 1923; England and Rice 1957; Warcup 1957; Witkamp 1960; Reddy 1962; Fincher 1963; Gams and Domsch 1969; and Moubasher and El-Dohlob 1972; and Moubasher and Abdel-Hafez 1978). They all reported periodicity in the composition of the fungal flora of the soil, but many observations were conflicting due to remote variations between localities studied. Most studies reported higher fungal abundance and diversity outside the summer months when soil temperatures were moderate to low (i.e., 10 to 25^0C). During the high temperature months (i.e., 34 to 38^0C) *Aspergillus* species are the most common species isolated from dry exposed sites (Moubasher and Abdel-Hafez, 1978). Given the high degree of successional turnover of microbial species exhibited on small scales, compositional instability seems to be a characteristic of many microbial systems. Given the generalist nature of most soil saprophytes, gross functional stability may not necessarily be linked directly to compositional stability.

When temporal variability and scale are considered, only then can we fully evaluate the relationship between diversity, stability, and functional similarity in microbial systems (Gaston 1996). At small spatial scales, it has been demonstrated that leaf litter decomposition rates are primarily regulated by community structure and micro-climatic conditions (Schimel 1995). Sugai and Schimel (1993) found little difference in rates of leaf litter decomposition between different successional groups of fungi in Alaskan forests, and suggested that microbial species were either functionally similar or ubiquitous saprophytes. It may be that attempts to identify microbial functional groups based on leaf litter decomposition rates is problematic due to the cosmopolitan nature of most cellulose degrading fungi, and the plethora of organisms capable of degrading plant material. Additionally, differences in microclimate and diversity of soil arthropods may influence leaf litter degradation rates as the plant community changes with succession. These types of confounding influences make it difficult to associate small scale variations in microbial species to large scale ecological processes.

In summary, plant species diversity seems to be related directly to fungal species diversity in forest ecosystems. Soil fungi extract scarce or bound nutrients and water from forest soils for plants, and protect seedlings and rootlets from disease and toxins (Atlas and Bartha 1998). Microorganisms are sources of particular compounds in ecosystems and sinks for others. The critical enzymatic activities involved in a particular elemental transformation within a habitat may be associated with one particular microbial population or with multiple microbial, plant, and/or animal populations. The important role mycorrhizal fungi play in plant community structure is widely acknowledged, and loss of biodiversity could lead to loss of crucial ecosystem processes. Greater understanding of microbial species compositions and function will allow for more efficient management and

restoration of modified habitats. However, the extent to which within-group diversity of microbes results in process heterogeneity or buffering against environmental change has yet to be determined (Gaston 1996; Debinski and Holt 2000). We know much about the formation of soils and leaf litter, their chemistry, and physics, and we recognise a multitude of soil organisms, however, there is a fundamental need to decipher the mysteries of microbial community structure and diversity and relate this to other components of biodiversity and wildlife health.

2) Disease and disease-causing agents

Definitions of 'health' and 'disease' vary greatly; therefore, it is important that I define these terms in the context of this thesis. The World Health Organisation defines health as "a state of complete physical, mental, and social well being and not merely the absence of disease or infirmity" (Last 1983). The term disease defines "any impairment that interferes or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxins, and climate; infectious agents; inherent or congenital defects, or combinations of these factors" (Wobeser 1981). These impairments can impact negatively on the long-term survival of wildlife and alter ecological roles in an ecosystem (Deem *et al.* 2001).

There are clearly a multitude of causal factors that can potentially contribute to the development of disease. Causes of disease can be simplified into non-infectious and infectious causal factors. Non-infectious diseases are caused by non-living factors such as poisons and toxins, genetic defects, traumatic events, and degenerative abnormalities. These are usually refered to as causative or risk factors (Hartup *et al.* 1998). Infectious diseases are caused by living organisms that inhabit either the external surface or the internal structures of the body. Viruses, bacteria, fungi, protozoa, arthropods and worms are all within this group, and are usually refered to as causative agents (Wobeser 1996). Infectious diseases can further be divided into contagious and non-contagious diseases. Contagious diseases are caused by living organisms that spread or are transmitted from one member of the population to another. The transmission of these diseases are dependent on contact between members of the host population, and occur more frequently in host populations of high density, e.g, tuberculosis. Non-contagious diseases are generally not transmitted from individual to individual. While the causative agent will infect animals and cause disease, it can also occur as a free-living organism within the same environment. Most non-contagious diseases-causing agents are not dependent on the host for growth or reproduction. Therefore, exposure and host susceptibility rather than host population density are more important in non-contagious *vs* contagious diseases, e.g., aspergillosis (Wobeser 1996).

In all infectious diseases the development of infection depends on exposure to the disease-causing agent/s, and the hosts ability to resist infection and disease (Wobeser 1981). Host resistance to disease can be highly circumstantial. Resistance to most non-contagious diseases is usually not a problem for healthy individuals providing exposure is not a primary causal factor leading to infection. For non-contagious diseases the initiation of an infection is usually considered to be opportunistic or secondary to other primary causal factors such as immunosuppression due to starvation, sickness, exposure to toxins, and/or psychological stress (Wobeser 1996). However, elevated exposure to some disease-causing agents such as *Aspergillus fumigatus* can be associated with both non-infectious and infectious type diseases in the host, e.g., aflatoxicosis and aspergillosis.

3) *Aspergillus and* aspergillosis

There is growing interest in the importance of small-scale disturbances (i.e., patch dynamics) on community structure and function in Conservation Biology (Meffe and Carrol 1994). Many species are dependent on disturbance for their survival, and would become extinct in stable or homogeneous environments. For these species, disturbances may function as refugia, windows of opportunity, or resources (Wilson and Peter 1988). *Aspergillus fumigatus* may be such a species that benefits from habitat disturbance, and like all weedy-pest species is found most commonly in modified environments such as forest edge habitats (this study). However, unlike most weed-like species *A. fumigatus* produces reproductive propagules that are pathogenic to most forms of life, and this poses a potential human and wildlife health risk in modified environments. At present, little is known on the role of *Aspergillus* when it is not infecting its host/s, other than the genera have broad environmental tolerances, and can lead an active saprophytic life on most elementary substrates found in nature (Atlas and Bartha 1998).

Because *A. fumigatus* spores can be distributed by global wind currents (e.g., Smith *et al.* 1996), most authors seem to credit *A. fumigatus* with having an almost omnipresent existence. However, this study provides direct evidence that suggests *A. fumigatus* is less abundant in mature forests compared to modified environments. *Aspergillus fumigatus* is a naturally occurring cosmopolitan fungus and common avian pathogen (Bauck 1994). *Aspergillus fumigatus* is the principal disease-causing agent for aspergillosis in birds, and has been identified as a primary mortality factor among free-living hihi on Mokoia Island (Alley *et al.* 1999). Aspergillosis occurs in acute and chronic forms. The acute form primarily occurs in young and recently translocated birds (Woodford and Rossiter 1994). The chronic form is more likely to occur in older birds that have been in captivity (Locke 1987; Schultz *et al.* 1996). *Aspergillus fumigatus* spores are widespread in modified

environments and many birds probably carry them in their lungs and air sacks until immunosuppression or stress triggers clinical disease (Bauck 1994). However, it is possible that some bird species are more susceptible to aspergillosis than others, and identifying these species and *A. fumigatus* habitats may allow us to better gauge habitat suitability for these species.

Exposure to aspergillosis usually occurs through the inhalation of fungal spores, but in healthy birds exposure does not always lead to disease (Coelho 1990). The notion that all healthy birds are essentially immune to aspergillosis is almost entirely based on epidemiological studies of the disease in domestic and captive birds under varying degrees of confinement. While there is little doubt regarding the opportunistic nature of aspergillosis in humans and domestic animals, it is dangerous to apply this reasoning to wildlife species. The immune system of any organism is complex, and a key question is whether current approaches are adequate given this complexity. For example, one could predict that if allergies represent a potential cost to aggressive immunity, then there may exist a positive correlation between the prevalence of allergies and habitat modification, and a negative correlation between prevalence of allergies and incidence of disease (Zuk 1994; Lochmiller and Deerenberg 2000). Therefore, the immunological systems of humans and domestic animals may have already made important adaptations towards resisting diseases more commonly associated with modified habitats, and this may give them greater resistance to diseases such as aspergillosis.

Aspergillosis has been diagnosed in a great variety of captive and free-living bird species. Aspergillosis is typically a disease of captivity and close confinement, particularly when birds are kept in unclean conditions (Keymer 1982; Woodford and Rossiter 1994). Some bird species appear to be more susceptible to aspergillosis than others, and this is demonstrated by the fact that the disease is especially common in seabirds when brought into captivity in a terrestrial environment (Bauck 1994). Likewise, some terrestrial bird species that have historically had little contact with modified habitats may suffer low resistance to aspergillosis as well. Evidence for this may exist in the types of acquired immunological adaptations exhibited within and between bird species surviving in these types of habitats. Alternatively, it is possible seabirds suffer higher states of immunosuppression when handled, and this may primarily cause their susceptibility to this disease. If we can achieve a greater appreciation of the connections between life histories and immunological adaptations that have occurred in domestic animals over the course of domestication and extrapolate this to wildlife populations being forced to occupy modified habitats, we may be able to partly resolve the issue of resistance *vs* susceptibility to aspergillosis.

Like physical and psychological stress, consumption of *Aspergillus* spores can lead to immunosuppression and a host of other diseases (Luckey 1965; Shimoda 1979; Coelho 1990). Aflatoxicosis is a disease caused by the naturally occurring mycotoxin aflatoxin, which is contained in the spores of several strains of *Aspergillus* species, including *Aspergillus fumigatus* (Chaudhary and Singh 1983). Large scale outbreaks of aspergillosis and aflatoxicosis have been reported where captive birds have been exposed to high densities of spores in their grain and seed feed (i.e., Chute and O`Meara 1958; Hamilton 1982; Robens 1990). Additionally, *Aspergillus* species are commonly isolated from the external surface of eggs following laying (Chute and Richard 1991). Spores of *A. fumigatus* can penetrate fresh or incubating eggs through the pores and grow on the shell membranes (Campbell 1986). Newly hatched chicks can then become infected in this manner (Bauck 1994). Autoimmune diseases such as aflatoxicosis can predispose birds, especially small birds, to aspergillosis without the need to evoke subjective psychological risk factors.

There is little doubt that physical and/or psychological stress factors can increase the risk of disease in a host. It also seems sensible to assume that domestic animals are more psychologically conditioned to human contact compared to free-living species, and these factors may make free-living animals susceptible to stress. Effects of psychological stress on the immune system has led to research linking the immune system with the nervous system (Zuk 1994; Lochmiller and Deerenberg 2000). This has some potentially interesting implications for connections between immunity and behaviour, and the possibility that the immune system can be primed by stimuli of the nervous system. In addition, life history differences and variations in immunocompetence may also relate to survival. A critical assumption of the evolutionary ecology paradigm is that variation in traits can evolve in response to selection. If so, what are the prospects for genetic variation in some of the trade-offs between immunity and other activities?

Aspergillosis is an infectious disease that can be caused by several species of *Aspergillus*, including *A. fumigatus* (Bauck 1994). Aflatoxicosis is a non-infectious autoimmune disease caused by the mycotoxin aflatoxin, which is also produced by several species of *Aspergillus,* including *A. fumigatus* (Robens 1990). However, while *A. fumigatus* is the most reported causative agent of aspergillosis in birds, it is not the most common causative species for aflatoxicosis in birds, that title belongs to *A. flavus* (Karup and Kumer 1991). Aflatoxin is one of the most carcinogenic naturally occurring substances known to man, and commonly contaminates feed and foodstuffs (Shimoda 1979). Mycotoxins are secondary metabolites produced by a multitude of saprophytic and parasitic fungi, and play an important role in regulating community structure (Atlas and Bartha 1998). Aflatoxin is contained within *Aspergillus* spores (conidia) which are usually either consumed or

inhaled into the respiratory system by the host (Pitt 1994). Elevated levels of mycotoxins in the diet and/or nesting environment could predispose parents and their offspring to immunosuppression and opportunistic diseases (Bauck 1996). This may be particularly applicable to small birds like hihi, which build their nests in damp cavities and have relatively long nesting periods. If there are costs and benefits associated with immunocompetence then becoming better adapted to fight off one pathogen might limit the host in responding to other pathogens (Sheldon and Verhulst 1996). By producing particular chemicals such as antibodies, MHC molecules, and lysozymes, a particular individual may then be constrained in the range of disease-causing agents they can resist (Lochmiller and Deerenberg 2000). The humoral system of vertebrates gets around this constraint to a large degree, but does it remove it entirely?

4) Potential role of aspergillosis in the decline of the hihi
There are 167 species of honeyeater (Meliphagidae) confined to Australia and the general Pacific region (Ford 1985). In Australia the family is represented by 72 different species, and is the dominant bird family in most habitats (Ford 1985, 1989). In New Zealand the family is only represented by three species, the hihi (or stitchbird*)*, tui (*Prosthemadera novaeseelandiae*) and bellbird (*Anthornis melanura*). Hihi are more sedentary than bellbirds and the tui (Craig *et al.* 1981).

The hihi is a slender starling sized bird (i.e., 30-45 grams). They are sexually dimorphic and have a variable mating system, including monogamy and various types of polygamy (Castro 1994a, 1996). The male hihi is more colourful and on average weighs several grams more than the cryptic female. Both sexes bear a prominent white bar on each wing and have features typical of nectar and insect feeding birds (Rasch *et al.* 1996. DOC recovery plan). Hihi feed on flowers, fruits and invertebrates in differing proportions from season to season (Gravatt 1970,1971; Angehr 1984, 1985, 1986; Rasch 1985; Rasch & Craig 1988; Castro 1994a, 1997; Perrott 1997).

The hihi from New Zealand and the O'o (*Moho spp.)* of Hawaii are the only honeyeaters known to build their nests in tree cavities. As in New Zealand, the native lowland forest in Hawaii have been almost completely cleared and its avifauna devastated by habitat change, introduced predators, competitors and disease (King 1984; Atkinson *et al.* 1995). Among bird species, cavity nesters and ground nesters have been the most strongly affected by these 'disturbance' events (Nice 1957; Lack 1968). Since European colonisation the hihi and O'o have both suffered dramatic declines. Hihi became confined to a small offshore island (Little Barrier Island or Hauturu), while three of the four

O'o species became extinct and the other is extremely rare (Coluzzi *et al.* 1979; Atkinson *et al.* 1995).

In pre-European times hihi occurred throughout the North Island; on Great and Little Barrier Islands and Kapiti Island (Oliver 1955). Evidence from North Island avian subfossil remains suggests hihi may have been more abundant in the lowland forests of the upper North Island (Millener 1981). While hihi sub-fossil remains have been found as far north as North Cape, they have not been discovered in the South Island. Fulton (1908) provides hearsay evidence that hihi were once common in the South Island, and were already in decline prior to the arrival of Europeans in New Zealand, e.g., "the stitchbird, according to the Maoris (were) once common throughout the islands, was rare on the arrival of the pakeha".

As hihi declined on the mainland there is evidence that they vanished first from the lowland forests (Buller 1888). This is not surprising, as lowland forests suffered the highest rates of deforestation in New Zealand during the nineteenth century (King 1984). Within the space of a few decades hihi had vanished from the mainland completely, even though there were still large tracts of primary forest on the mainland at the time. At the time, mature forests still extended virtually unbroken from the Thames Mountains, to Rotorua, almost meeting the great forests that covered the whole East Cape region, and then following the Ruahine and Tararua Ranges to the Cook Strait (Fulton 1908). In addition, during this period hihi were reported to be declining on Little Barrier Island, which may further suggest disease as a possible causal factor for their decline (Reischek 1881,1886). It has been suggested that this disease could have been brought into New Zealand with the introduction of exotic bird species during the later part of the nineteenth century (i.e., Angehr 1984; King 1984). Alternatively, it is possible that the disease causing agent/s were already present, and the relationship between deforestation and disease is more than coincidence.

Attempts to establish hihi populations on other islands from Little Barrier Island in the 1980s were unsuccessful (Rasch *et al.* 1996). The main explanation given for these early failures were that small islands probably had an insufficient year-round supply of nectar and fruit sources as a result of habitat modification (Castro 1994a,b). Hihi were subsequently translocated to another small island during 1994 (Mokoia Island, 135 ha) to test this hypothesis (i.e., Armstrong and Perrott 2000). Armstrong and Perrott (2000) experimentally tested the carbohydrate-limitation hypothesis in the first year of the hihi release on Mokoia with negligible results. We unexpectedly found that birds on average tended to gain about 1.1 g when feeders were removed. However, the annual hihi survival rate was about 40%, both in the year of the experiment and in subsequent years, and the

population has continued to decline. Armstrong and Perrott (2000) concluded that the viability of the Mokoia hihi population is limited by a low survival rate and that this is unrelated to food availability. They further suggested that declines of other translocated hihi populations were unrelated to fruit and nectar availability. During this time examination of sick and dead hihi collected on Mokoia from 1995 to 1997 revealed that aspergillosis was the primary causal factor for the high mortality rate among birds sampled (Alley *et al.* 1999). Captive hihi at the Mt Bruce Wildlife Centre are also known to be susceptible to aspergillosis. It is possible that the food-supplementation experiments carried out on Mokoia were confounded by the poor health and high levels of aspergillosis among birds in the study. High levels of disease among male hihi on Mokoia may have reduced their ability to gain weight when fed supplementary food, and this could have obscured our ability to identify food shortages in the hihi population on Mokoia. There is tentative evidence to support this notion as male hihi on Mokoia weighed on average 3.4 g lighter than their counterparts on Tiritiri Matangi Island (unpublished data). However, weight differences between female hihi varied little between the two populations. Nevertheless, the situation with hihi on Mokoia Island and at the Mt Bruce Wildlife Centre indicate that ensuring birds have a good supply of food and nest boxes is little insurance against aspergillosis in such environments.

During 1996/1997 Armstrong and Ewen (2001) repeated the same food-supplementation experiments with hihi translocated to Tiritiri Matangi Island, and found that hihi survival was negatively effected by seasonal limitations in natural food supplies. Additionally, Castro *et al.* (In press) studied the effects of food supplementation on egg laying and incubation in hihi on Mokoia Island from 1995 through 1997, and found that food supplementation significantly increased clutch size, reduced incubation times, and increased hatching success of most breeding birds in the study. From this they suggest that female hihi are probably food limited during the nesting period, and that the successful establishment of hihi populations on islands with poor natural food supplies, such as Mokoia, may be dependent on the permanent provision of supplementary food at the nest site of each breeding hihi female. These results directly indicate that the quality of the natural food supply on Mokoia is unsuitable to hihi, and that this could be limiting hihi fecundity on the island. However, this does not necessarily imply that there is a food availability problem on Mokoia; it does imply that foraging from artificial feeders is more profitable to a breeding hihi than foraging from natural food sources. Additionally, if birds are sickly and having trouble gaining weight in the first place, providing supplementary food outside hihi nest boxes may allow birds to better maintain a healthy body weight. Additionally, providing supplementary food outside hihi nest boxes seems to reduce the time birds spend foraging and sitting on the nest, which may suggest that artificial feeders allow birds to better time-budget disease avoidance behaviours such as personal hygiene and

sunning activities. If the natural food supply on Mokoia is unsuitable to hihi for reasons other than availability, i.e., such as low nutritional quality and/or contaminated with toxins, then food supplementation may allow hihi to avoid consuming contaminated foodstuffs. Additional evidence supporting food supplementation on Mokoia was observed during the 1998/1999 breeding season. During that breeding season no food supplementation was provided for hihi, and no fledglings survived from that year (*pers comm*- Dr D.P. Armstrong).

To date, little has changed for hihi on Mokoia Island. The hihi population is still in decline; fledglings still suffer high mortality rates, and seldom survive past their first two years. Coordinated research over seven years on Mokoia has implicated two major factors responsible for this decline, 1) poor quality of the natural food supply available to breeding females, and 2) fungal disease aspergillosis. This thesis will suggest that both factors could be related to the high prevalence of *A. fumigatus* on Mokoia, and that feeders basically provide hihi with the opportunity to die from chronic aspergillosis instead of acute aspergillosis. Additionally, the lower *A. fumigatus* counts on other islands with resident hihi populations may account for the lower mortality rates among hihi on those islands. If food limitation is the principle causal factor for the decline of hihi on Mokoia, why do hihi continue to show signs of aspergillosis and vanish on Mokoia Island? Why do well cared for hihi suffer such high rates of aspergillosis at the Mt Bruce Wildlife Centre? If nothing else, the situation on Mokoia illustrates that food supplementation experiments designed without wildlife health components incorporated into them lack explanatory power.

5) Thesis plan

The primary aim of this project was to investigate whether hihi translocations from Little Barrier Island to young growth regenerating forest islands such as Mokoia were exposing hihi to elevated levels of *Aspergillus fumigatus*, and therefore, whether *A. fumigatus* preventative management should be considered when planning future hihi translocations. It is presently not possible to correlate natural variations in *A. fumigatus* densities with the survival and/or fecundity of any free-living bird species. Therefore, it was important to obtain good data on the natural occurrence of *A. fumigatus* on islands with surviving hihi populations to increase our understanding regarding why hihi on Mokoia and at the Mt Bruce Wildlife Centre seem to be particularly susceptible to aspergillosis (Alley *et al*. 1999). I intended to address the question of whether elevated exposure and low resistance to *A. fumigatus* could partly explain hihi susceptibility to aspergillosis, as opposed to subjective factors such as stress and health. While few people would argue with the idea that translocations are a particularly stressful time for any animal, whole generations of hihi continue to vanish every two to three years as a result of this disease on Mokoia Island.

My results in this thesis are reported in Chapters 2 through 4. Chapter 2 describes the abundance and distribution of native cicadas, and their associations with entomophagous fungi on Mokoia Island and at the Eastwoodhill Arboretum. Data from this chapter is used in other chapters to estimate *A. fumigatus* counts and soil turn over rates associated with cicada emergence on Mokoia. Chapter 3 describes temporal changes in *A. fumigatus* in the surface soil and response to leaf litter removal. Chapter 4 describes *A. fumigatus* densities in the soil, leaf litter, floral nectar, and air in relation to forest succession and forest edge habitats on Mokoia Island, Tiritiri Matangi Island, Little Barrier Island, Mt Bruce Wildlife Centre, and the Massey University Palmerston North Campus. All study sites mentioned, other than the Massey University Campus site, were selected because these sites had resident hihi populations. Chapter 5 is concerned with comparing the microclimate and *A. fumigatus* densities between hihi nest boxes on Mokoia Island and puriri tree cavity nests on Little Barrier Island. Chapter 6 is a general discussion concerning potential global implications of results from chapters 2 through 5.

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Chapter 2

Cicada densities and associations with mycopathogens on Mokoia Island (Lake Rotorua), at and Eastwoodhill Arboretum (Gisborne) New Zealand.

Introduction

Cicadas represent a seasonally abundant, nontoxic, easily captured prey, and are consumed by a wide range of animals (Beamer 1931; Brown and Chippendale 1973; Lloyd and Dybas 1966). Cicadas are found in virtually all terrestrial habitats, from seaside dunes and riverbeds to alpine zones; from rank roadside grass to dense primary forest. Nevertheless, cicadas do exhibit habitat preferences and are particularly attracted to forest edges, exposed bush aspects, and new growth forests (White 1980; Williams and Simon 1995). Mokoia Island (Lake Rotorua) is a young growth forest island, and cicadas are a prominent component of that habitat.

As mentioned in Chapter One, hihi (or stitchbird, *Notiomystis cincta*) on Mokoia are commonly observed feeding their chicks on cicadas, and at times discard cicada parts within the nest box. Discarded cicada parts with visible fungal growths have been removed from active hihi nest boxes on Mokoia. Hihi are known to be susceptible to the fungal disease aspergillosis (e.g., Allley et al. 1999), therefore, there is concern that hihi are contaminating their nest boxes with *A. fumigatus* while carrying out their parental duties. This chapter reports work designed to measure cicada densities and identify mycopathogens associated with dead cicadas on Mokoia Island and at Eastwoodhill Arboretum. The arboretum has no resident hihi, but does have a resident cicada pest problem, and is included in this thesis to test independent cicada density estimation methods at two known cicada hotspots. Mokoia cicada density data is later used to estimate soil turnover rates caused when cicada nymphs emerge from their subterranean burrows, shed their last exoskeleton, and develop into winged adults. This data is later extrapolated with additional data from chapters three and four to estimate associated *A. fumigatus* turnover rates per unit area of soil during the cicada emergence period.

Therefore, this chapter represents preliminary data used in other chapters, and is primarily concerned with, a) methods testing Mokoia cicada census methods by attempting to replicate sampling procedures at EWH, b) identifying and estimating levels of fungal disease in the cicada population on Mokoia, and c) reporting the occurrence of a new fungal disease observed fixing adult cicadas to vegetation on Mokoia Island.

Previous studies on New Zealand cicadas have been mostly descriptive, detailing species distributions and song characteristics. Experimental ecological studies of New Zealand cicadas are rare (e.g., Cumber 1952; Fleming and Scott 1970). An equally small number of studies have been concerned with estimating cicada densities and hybridisation rates in New Zealand (e.g., Barratt 1983; Lane 1984; White and Sedcole 1993). All ecological studies from the North Island have been concerned with the genus *Kikihia* (e.g., Cumber 1952, Fleming and Scott 1970, Lane 1984). South Island studies have been concerned mostly with the subalpine cicadas of the genera *Kikihia, Maoricicada*, and *Rhodopsalta* (e.g., Barratt 1983, White and Sedcole 1993). Other than fungus-cicada nymph interactions, such as the "vegetable-cicada" *Paecilomyces cicadae*, fungus-cicada interactions have received little attention in New Zealand.

Cicadas make good candidates for studies on entomophagous fungi because they spend the majority of their life cycle underground as nymphs, and prolonged development or contact with the soil favours the occurrence of fungal diseases (Ferron 1978; Irwin and Coelho 2000). As far as I can ascertain no Entomophthorale diseases have been reported in any New Zealand cicada species prior to this study. One Entomophthorale fungus from the genus *Massospora* has been reported in Australia infecting mature cicadas, but this fungal parasite has not been identified in New Zealand cicadas (Soper 1978, Max Moulds *pers comm*). This study reports an entomophthoran cicada disease caused by an unidentified *Conidiobolus* species that exhibits sporulation and altered behaviours in its cicada host prior to death. This unique cicada disease is included in this chapter to contribute to the developing knowledge base from which to compare host-pathogen systems and discover commonalities.

In the literature there is a multitude of entomopathogenic fungi that demonstrate an ability to have significant impacts on their host populations. However, few insect-fungi

interactions have been studied in great detail (Hajek and St. Leger 1994). Investigations on interactions between soil insects and their mycopathogens are particularly difficult and time consuming. The majority of existing studies are restricted to examples of commercially or medically important fungal pathogens. At present, scarabaeid and curculionid larvae are the principle targets for microbial control. To understand more about these insect-fungus interactions ecological studies on the natural occurrence and distribution of mycopathogens in different soil types and in different geographical regions independent of specific target insects are needed (Keller and Zimmermann 1986).

With the exception of a *Condiobolus* species, all fungi found infecting cicadas during this study can be cultured on artificial media. Strictly, therefore, they are all faculative pathogens and/or saprophytes. The unidentified *Conidiobolus* species was observed fixing mature cicadas to vegetation on Mokoia Island in late February 1999. Prior to death the infected cicadas become lethargic, and easily caught by hand. If left undisturbed the infected cicada grasps onto a vertical surface like a twig or tree trunk, making a "death-grip" with its first two pairs of legs, then inserts its rostrum into the plant as though feeding. Shortly thereafter, the cicada dies and hyphae emerge from between the sclerites along the ventral surface of the cadaver attaching it securely to the vegetation. The cadaver breaks down slowly in this position over several weeks with finally only the head and rostrum remaining attached to the vegetation. All cicada species identified on Mokoia were susceptible to this disease. The disease pattern has similarities to the "summit disease" syndrome reported in crickets and grasshoppers, typified by the Entomophthorale fungus, *Entomophaga grylli*.

Entomophthorales, such as the allied *Conidiobolus* species invade mature insects, and have a brief parasitic phase with death usually occurring within hours. Only the least specialised members of the Entomophthorales produce mycotoxins. These species all belong to the genus *Conidiobolus*, which tend to have wide host ranges (Prasertphon and Tanada, 1969). In the Entomophthorale group there is a progressive advancement from sporulation shortly after the death of the host to sporulation on the still living insect. Rhizoids are specialised hyphae that enable the mycopathogen to attach their host insect to a surface, sometimes with the aid of adhesive substances. This prevents the cadaver from falling to the ground where it might be removed by scavengers or

degraded by rain and/or faster growing opportunist fungi. Other factors are also likely to be associated with the disappearance of cadavers on the ground. Slugs are commonly associated with insect cadavers, perhaps feeding on fungal spores as slugs are omnivorous and known to consume fungi (South 1992).

Obligate insect parasites such as *Massospora cicadina,* which have a highly developed parasitic phase, the importance of mycotoxins and rhizoid formations tend to diminish (Balazy 1984). Invasion and destruction, rather than mycotoxins, cause host death. The older, diseased cicadas usually return to and die in the soil, which becomes contaminated with resting spores that plague the next generation of emerging cicada nymphs. For this group of fungi, which comprise the majority of the Entomophthorales, the saprophytic phase is minimal or completely absent. *Massospora* are the only known mycopathogens confined to cicadas. Infection in the mature cicada is restricted to the distal abdominal segments. The infected cicada slowly starves to death, but remains alive for several days. Primary sporulation usually occurs on the living host, which acts as a mobile dispersal vehicle for the fungus. As the disease progresses the abdominal segments, packed with *Massospora* spores, periodically fall from the cicada. While the cicada is in flight, the wings brush against each side of the abdomen spraying spores with every stroke. This leaves a distinctive white patch on the inside of each of the front wings. Spore patches were also evident on some cicadas infected with *Conidiobolus* from Mokoia Island.

Prolonged survival is probably better when the pathogen is adapted to a combined parasitic/saprophytic existence of the kind known for most *Conidiobolus* species (Papierok, 1985). This will be important for the survival of the mycopathogen, particularly when primary hosts are absent (Keller and Zimmermann 1986). However, an adaptation to saprophytism, i.e., mycotoxin production, may concomitantly impair pathogenicity. In some cases, the primary fungal pathogen may kill its host too quickly and be out competed by secondary faster-growing saprophytic fungi. For example, *Mucor* and *Aspergillus* can quickly overgrow insects already infected with fungi such as *Conidiobolus* (Papierok, 1985).

Entomophthoran fungi are more commonly reported infecting Lepidoptera from undisturbed habitats, while Deuteromycete fungi such as *Beauveria* are more commonly

found infecting soil insects in pasture and orchard habitats. Baker and Baker (1998) screened for generalist entomophagous fungi in pasture, cropland and two indigenous forests. Using *Galleria* larvae baits they reported higher *B. bassiana* infection rates in pasture soils than soils from forest samples.

Ascomycetes and Deuteromycete fungi lack resting spores, and kill their prey with toxins living saprophytically on the cadaver. These are the *Cordyceps and Paecilomyces* infections and the muscardine diseases (e.g., *Beauveria* and *Metarhizium*), each with several hundred species known. In New Zealand the "vegetable caterpillar" and "vegetable cicada" are two distinct species, *Cordyceps robertsii* and *Paecilomyces cicadae (=C. sincliarii)* respectively (Dingley 1974). *Paecilomyces cicadae* infects fifth instar cicada nymphs of the genera *Amphipsalta* and *Kikihia*, very few attack mature cicadas. It is not clear how *P. cicadae* infections arise, however, it is likely that the fungus gains entry through the respiratory system in the days/weeks prior to emergence. The cicada is not entirely mummified; in most cases the fungus forms a colony of white mycelium around the cicada. During Autumn *P. cicadae* grows a 2-8 cm stalk from the head of the cadaver. This releases clusters of minute white asexual spores above ground.

From the cicada density and the fungal screening results we answer the following questions: 1) What is the diversity of fungi infecting cicadas at each study site? 2) What proportion of the adult cicada population is infected with fungal pathogens? 3) What proportion of the nymphal populations have fungal infections? 4) How does the frequency and diversity of cicada mycopathogens vary between cicadas caught alive and those found dead on the ground, and/or attached to vegetation? It should be noted that this study gave no indication as to the extent of intragenera diversity, which can be considerable in fungi.

Field Methods

Eastwood Hill Arboretum (EWH) contrasts markedly with Mokoia Island. Apart from being a large mainland site (about 800 ha), EWH is a mature composite forest composed of exotic trees species, many 60 years old. Hundreds of sheep are periodically grazed between the trees and patches of forest at the arboretum, and thousands of visitors walk

the established paths throughout the year. Mokoia is a small (135 ha) forested island composed mostly of regenerating native tree and shrub species. Like EWH, Mokoia, during the 1920s, was farmland. A variety of domestic stock was farmed on Mokoia until the 1950s. Over the last 40 years secondary forest has spread from the gullies and now covers most of the island. The subcanopy regeneration, however, has occurred since 1989 when goats and rats were eradicated. The ground cover at EWH is mostly open grassland, while on Mokoia the forest floor has a thick covering of leaflitter.

Density and distribution of cicada exoskeletons

This project was started in September 1998 at Eastwood Hill Arboretum (Gisborne) and Mokoia Island (Lake Rotorua). The cicada emergence period for all species started during November 1998 and concluded in March 1999 at both study sites. During this period I visited each study site on a fortnightly basis collecting mature cicadas for density estimates and fungal screening. Outside the cicada emergence period (April 1999- October 1999) I visited each study site on a monthly basis collecting cicada nymphs and exoskeletons for density estimates and fungal screening. Sampling for exoskeletons were conducted monthly at fixed sample sites, emergence traps, and from random sample points taken along transect lines.

1. *Fixed sampling areas*

At EWH density estimates using eight fixed 1 m radius exoskeleton sampling areas were taken monthly throughout the emergence period, and again one year following the cicada emergence period. These eight 3.14 m^2 sample areas were selected prior to the 1998/99 emergence period by griding both study sites and randomly selecting eight grids. Each grid then had one fixed sampling area set-up at its centre. All fixed sites were placed around the nearest tree trunk and marked out with pegs and string, and all old exoskeletons from the year before were removed.

On Mokoia Island eight fixed sampling areas were initially set up, however there were too many exoskeletons appearing to count in the time available. To correct this problem I abandoned all fixed sampling areas except the first one which was located in a bush patch in the centre of the old horse paddock. This fixed sampling site was then extended to 6.3 m^2.

A fixed sampling area included the area beneath the canopy of the study tree/s, their trunks and branches. Every exoskeleton found in the fixed sample areas were individually marked with coloured paint. The colour of the paint changed with the month the exoskeleton was found. A peg marked the spot an exoskeleton was first found, and a record of the date. During each month of the emergence period new exoskeletons were marked and recorded. I recorded: a) the number of cicada exoskeletons per sample area, b) the monthly emergence rate, c) the sex and species of cicada the exoskeleton came from, and d) the proportion of exoskeletons remaining after one year.

After one year the remaining exoskeletons were collected and counted, and the proportion of exoskeletons remaining from the whole sample calculated. These data were then used as a function in the analysis of the random exoskeleton samples to estimate the proportion of exoskeletons from the previous summer (see random exoskeleton counts). In doing so I assumed that all exoskeletons regardless of species breakdown or go missing at similar rates.

2. *Adult cicada emergence traps*

At EWH 30 triangular mesh traps (0.4 m^2 each) were placed at each sampling site along transect lines. Selection of the transect lines involved measuring a distance of 25 m from the first 6 fixed exoskeleton sampling sites in a northern direction, and placing five traps at 5 m intervals along these lines. The traps were secured to the ground at these fixed locations for the duration of the cicada emergence period (Nov 1998- March 1999). The primary function of these traps were to collect recently emerged cicadas and their exoskeletons for emergence rate and density estimates. However, traps also supplied adults for dissection and screening for one-day-old cicadas (see laboratory methods).

On Mokoia 16 emergence traps were placed along two transect lines at 5 m intervals in the bush patch in the centre of the horse paddock. The two transect lines were 10 m apart and ran parallel along the length of the bush patch in a north/south direction from the fixed sampling site. I recorded: a) the number of adult cicadas, and therefore exoskeletons per trap, b) the sex and species of cicada, and c) distance of nearest tree/s within 2 meter radius. Adult cicadas collected from the traps were enclosed with their

exoskeletons in sterilised containers for transport back to the laboratory at Massey University (see below laboratory methods).

3. *Random exoskeleton counts*

I started collecting random exoskeleton samples in March 1999 and finished during July 1999 at both study sites. Random exoskeleton sampling was done at the sample points created whilst sampling for cicada nymphs (see nymph sampling). Twenty random exoskeleton samples were made every month at both study sites. At EWH I used a compass and measuring tape to locate 20 sample points along 4 transect lines during each month. The start point of each transect line was selected from 4 fixed sampling sites, alternating between odd and even numbered fixed sampling areas each month. Once the starting point was established, I measured 25 m from the starting point at a specific compass bearing. Samples were taken along this line at 5 m intervals. At each sample point I placed a 1 m^2 quadrat on the ground and counted all exoskeletons within that sample area. These included exoskeletons on vertical perches and under leaf litter. Once this was done I subtracted the estimated proportion of one-year-old exoskeletons from every random exoskeleton sample (see fixed sampling areas). This was done to calculate the approximate density of remaining exoskeletons from the 1997/98 emergence period, and therefore to calculate the number of exoskeletons for the 1998/99 emergence period.

On Mokoia the 20 monthly random exoskeleton samples were selected using a griding method. The island was divided into 28 grids. Each month I randomly selected 20 grids, using a compass and measuring tape I selected a sample point within each grid. The sample point within each grid was determined by measuring a specific distance from the central grid point at a specific compass bearing. If the random sample point was in the lake, I disregarded that sample, therefore, there was no bias for or against lake-edge habitat. I recorded: a) the number of cicada exoskeletons per sample area, b) the sex and species of cicada the exoskeleton came from, and d) the distance of nearest tree/s within 2 m radius

4. *Density and distribution of cicada nymphs*

Nymph sampling started in April 1999 and was completed during August 1999 at both study sites. Using a spade I dug 20 nymph sample holes a month at sample points

selected using the same methods as the random exoskeleton counts. A standard hole, one spade width in diameter (18 cm), was dug to a depth of 70 cm. All soil was removed and sieved by hand and replaced. I recorded: a) the number of cicada nymphs per surface area of soil, b) age of nymph to nearest instar, c) distance of nearest tree/s within 2 meter radius. Nymph samples were enclosed in sterilised containers for transport back to the laboratory at Massey University (see laboratory methods). Nymphal instars were identified using the descriptions provided by Marlatt (1909) and Cumber (1952). Adult cicada species were identified using descriptions provided by Fleming (1975b; 1984), Dugdale (1972), and Lane (1984).

5. *Collection of non-random adult cicada samples*

Throughout February 1999 I collected adult cicadas at EWH and Mokoia Island for fungal screening. This was done using an insect net on the end of a 1.8 m pole, and collecting cicadas caught in fixed emergence traps. Using the hand net with my arm at full stretch I could reach cicadas in trees perched below 3.7 m. All cicada samples collected were placed individually into sterile glass containers. Samples were then transported back to the laboratory at Massey University. I collected cicadas in four sampling categories. The first category is adult cicadas caught alive on vegetation. The second is adult cicadas found dead on the ground. The third category is adult cicadas found dead fixed to vegetation, and the forth category are cicada nymphs. All cicada samples were dead at the time they were screened for fungi. This was done to allow for the development of any disease patterns that might occur while the cicada is still alive and/or near death.

All cicada samples taken back to the laboratory were kept in their containers at 20°C till death. Each sample was then examined for conidia and/or resting spores.

Laboratory Methods

1. *Fungal screening of adult cicadas*

a) *Examination*: All cicada samples were examined externally and internally. The external examination involved recording the presence/absence of any injuries, fungal

material (mycelium & spores), and small arthropods. This information was recorded on a work sheet for each cicada sample. Fungal material was extracted for identification (see part b).

b) *Extraction*: All fungal material found on/in any cicada samples during the examination stage was directly extracted and examined under a microscope. I extracted fungal material by using a wire loop and/or scalpel to crop/scrape hyphae from the sample. Once fungal material was extracted the rest of the cicada sample was placed back into the sample container and stored in a fridge at 4°C. Cicadas not exhibiting mycoses upon death were sampled for fungi by rubbing the cicada along a microscope slide.

c) *Identification*: Once suitable cultures had been grown on cadavers or selective media, hyphae and reproductive bodies were extracted and mounted onto microscope slides. Using dyes to highlight the hyphae and reproductive bodies, photographs were taken for identification purposes. Using taxonomic keys most fungi were then classified to genera with the help of Hugh Nieelson (Institute of Natural Resources, Massey University), Dr Travice Glare (AgResearch, Christchurch), and Dr Mike Baxter (Microbiology Dept, Massey University).

2. *Fungal screening of cicada nymphs*

All nymphs found either dead and/or exhibiting mycosis during the nymph sampling program were placed individually in sterile containers and examined back at the laboratory, in most cases, two to three days later. In total, 114 nymphs were examined at EWH, and 320 nymphs from Mokoia Island.

a) *Examination*: All cicada nymph samples were examined externally for fungi using a dissection microscope. All fifth instar nymphs were examined internally as well. The external examination involved recording the presence/absence of any fungal material (mycelium & spores), and small arthropods. This information was recorded on a work sheet for each cicada nymph. Fungal material was extracted (see part b) for identification.

The internal examination of the fifth instar nymphs also involved recording the presence of fungal material, and evidence of other parasites in the abdomen. The nymph was dissected in the same manner as the adult cicada samples (see 1a). All samples were then examined for 'milky' haemolymph, a symptom of developing resting spores.

b) *Extraction*: All fungal material found on/in any cicada nymph samples during the examination stage were directly extracted, and placed onto a microscope slide (see next section). The methods for this part follow the same as those used for adult cicada samples (see above).

c) *Identification*: An attempt was made to identify all moulds found growing from nymph samples. Identification to genera of fungal material was done using taxonomic keys.

All results are expressed as the percentage frequency of occurrence. The statistical significance of observed differences between species and sex ratios are made using Chi-square test.

Results

Two cicada genera were found to dominate at both study sites, these were *Amphipsalta* and *Kikihia*. At EWH there were two *Amphipsalta* species (*A. cingulata* and *A. zelandica*), and several *Kikihia* species, the most common being *K. muta*. Mokoia had the same *Amphipsalta* species, but the dominant *Kikihia* species was *K. cutora*.

The first male cicadas to emerge for 1998 at EWH were heard on the 3rd November. On Mokoia the first cicadas were heard on the 6th November 1998. At both study sites the first cicadas to appear were the cicadas of the genera *Kikihia*, and the small *A. cingulata*. These species were found mostly away from the forested areas at EWH and Mokoia, seeming to favour the ground and areas of tall grass, reeds, and flax.

The large cicada species *A. zelandica* was the most prominent cicada species at both study sites, mainly because of their size and loud song. This species started to emerge

during late December 1998 at both study sites, reaching peak numbers about four weeks later during January 1999. In total the emergence period lasted about two months with most *Amphipsalta* cicadas dying *en mass* around late February 2000.

1. *Cicada densities and distributions*

A) *Fixed sampling areas*

At EWH the selection of the sites for the 8 fixed sampling areas (FSA 1-8) were completed during October 1998. No exoskeletons were found at any of the EWH fixed sampling areas till late December 1998 (Fig. 2.1a). These were all *A. cingulata* species. Most cicadas that emerged at the EWH fixed sampling areas were found during January and February (239/247), and most of these were *A. zelandica* (93%). This gave a combined mean density of 10 emerging cicadas per square metre at EWH (SE= 4.0, Table 2.1). The emergent sex ratio at EWH was significantly biased towards males cicadas (P = 0.05), 46/247 exoskeletons coming from male cicadas (59%), and 101/247 exoskeletons derived from female cicadas (41%).

The density of cicadas that emerged at the fixed sampling area in the bush patch on Mokoia was significantly higher (i.e., nine times greater) than the mean density recorded at EWH (P = 0.02). On Mokoia a total of 588 exoskeletons were counted in the 6.3 m² fixed sampling area, which gave a density of 93 emerging cicadas per square metre. Of these 314/588 were from male cicadas (53%), and 276/588 were from female cicadas (47%). However, in contrast to EWH no *A. zelandica* were recorded at the fixed sampling area on Mokoia. The majority of the cicadas that did emerge in the Mokoia fixed sampling area were *A. cingulata* (79%), with the rest being cicadas of the genera *Kikihia* (21%). The first cicadas began to emerge in the Mokoia fixed sampling area during November 1998 and the last was found in April 1999 (Fig. 2.1b). This gave *A. cingulata* and *Kikihia* spp a six-month emergence period on Mokoia. The number of emerging cicadas at the fixed sampling area peaked during December 1998 (245/588), two to three weeks after the first emergent cicadas were recorded. In total this gave *A. cingulata* and *Kikihia* spp densities of 74 and 19 emerging cicadas per square metre.

Of the 247 exoskeletons recorded from the EWH fixed sampling areas, 52 of them were still attached to a trunk or branch by the end of April 1999 (21%). Of the 588 exoskeletons recorded from the Mokoia fixed sampling area, 200 of them were still

CICADA POPULATION DATA FOR EASTWOODHILL ARBORETUM AND MOKOIA ISLAND

November 1998- August 1999

	Eastwoodhill	Mokoia Island
Cicada spp	*Amphipsalta zelandica* *Amphipsalta cingulata*	*Amphipsalta zelandica* *Amphipsalta cingulata* *Kikihia* spp

A) Fixed sampling areas

8 sample areas (total area 8 X 3.14 m^2)		1 sample area (total area 6.3m^2)	
n	247 exos	n	588 exos
ave per sample area	31±12 exos	ave	NA
mean density	10±4 per m^2	mean density	93 per m^2

B) Emergence traps

30 traps (total area 30 X 0.4 m^2)		16 traps (total area 6.4m^2)	
n	99 exos	n	489 exos
ave per trap	3±1 exos	ave per trap	29±4 exos
mean density	8±2.4 per m^2	mean density	76±5 per m^2

C) Random counts

100 samples (total area 100 X 1 m^2)		100 samples (total area 100 X 1 m^2)	
n	369 exos	n	1179 exos
ave per quadrat	3.7±1 exos	ave per quadrat	12±2 exos
mean density	3.7±1 per m^2	mean density	12±2 per m^2

D) Nymph sampling

100 sample holes (100 X 0.032 m^2)		100 sample holes (100 X 0.032 m^2)	
n (instars 2-5)	114 nymphs	n (instars 2-5)	702 nymphs
ave per hole	1±0.5 nymphs	ave per hole	7±2 nymphs
mean density	35±5 per m^2	mean density	217±23 per m^2
n (5th instar)	77 nymphs	n (5th instar)	320 nymphs
ave per hole	0.8±0.3 nymphs	ave per hole	3±1 nymphs
mean density	24±5 per m^2	mean density	99±10.5 per m^2

Table 2.1. Combined totals for cicadas population census methods at Eastwoodhill Arboretum and Mokoia Island. Errors are calculated from standard error.

Cicada densities at 8 Fixed Sample Areas at Eastwoodhill Arboretum

A)
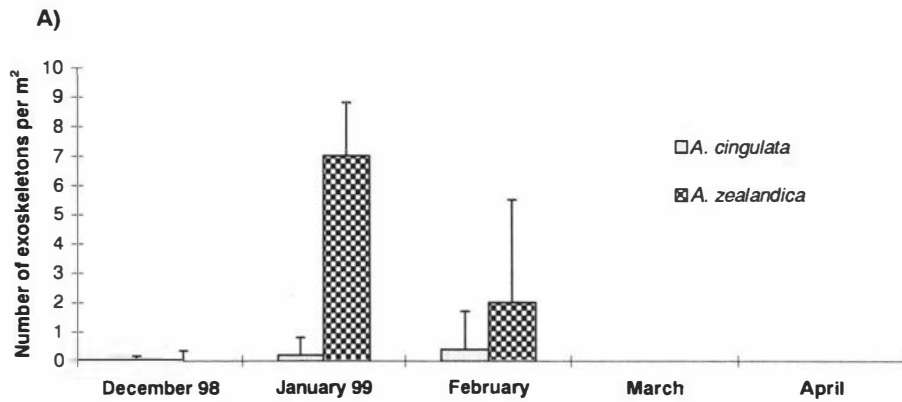
Cicada densities at one Fixed Sampling Area on Mokoia Island

B)
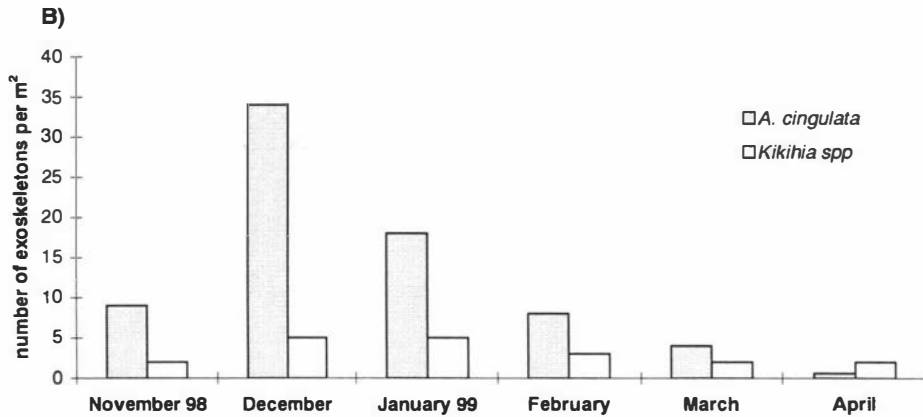
Figure 2.1. A) Density of *Kikihia* spp, *A. cingulata* and *A. zelandica* emerging each month from 8 Fixed Sample Areas at Eastwoodhill Arboretum (n= 247, total area sampled 25 m^2). B) One fixed sampling area on Mokoia Island (n= 588, total area sampled 6.3 m^2). Bars indicate standard error for figure 2.1A.

and A. zelandica (1%). The overall sex ratio on Mokoia was also biased towards males (P< 0.05), 293/489 exoskeletons were from male cicadas (60%), and 196/489 were from female cicadas (40%).

The mean timing of *A. cingulata* emergence from the Mokoia traps differed by one month compared to the fixed sampling area. While the cicadas that emerged in the fixed sampling area (6.4 m^2) were all probably feeding from the same mahoe tree, the cicadas that emerged in the traps were feeding from several tree/bush species spread over 200 m^2. This implies that *A. cingulata* nymphs feeding from the same host may more closely coordinate their development and emergence compared to single age broods feeding from different tree/bush hosts.

C) *Random exoskeleton counts*

Each month from March to July 1999 I took 20 X 1m^2 quadrat samples along transect lines at both study sites. Figure 2.3a outlines the mean monthly densities for the *A. zelandica* exoskeletons we found during the random counts at EWH. To better estimate the actual density of emerging *A. zelandica* at EWH, I subtracted the proportion of exoskeletons that were likely to have persisted from the year before. The whole-exoskeleton persistence score was calculated from the eight fixed sampling areas (EWH whole-exoskeleton persistence score= 26%, SE= 3.7). After subtracting 26% of all exoskeletons from each random quadrat I was left with 369 exoskeletons from 100 quadrats. This gave a mean density of 3.7 exoskeletons per square metre (SE= 1.0). The overall sex ratio was 196/369 male exoskeletons (53%), and 173/369 female exoskeletons (47%) (P < 0.05). Derived counts per quadrat ranged from zero to 90 exoskeletons per square metre. Most exoskeletons were found in the first two months (March and April) of sampling (213/369). By July, five months following the conclusion of the emergence period, exoskeleton counts were down more than three fold. From the exoskeletons found at EWH 224/369 were found attached to a tree trunk and/or branch (61%), even though only 15/100 quadrats had one or more tree trunks in the sample area (15%). This bias in their distribution is caused by the emerging nymphs seeking out vertical surfaces to eclose on, and further increases their already patchy distribution.

30 Fixed Emergence Traps at Eastwoodhill Arboretum

A)

16 Fixed Emergence Traps on Mokoia island

B)
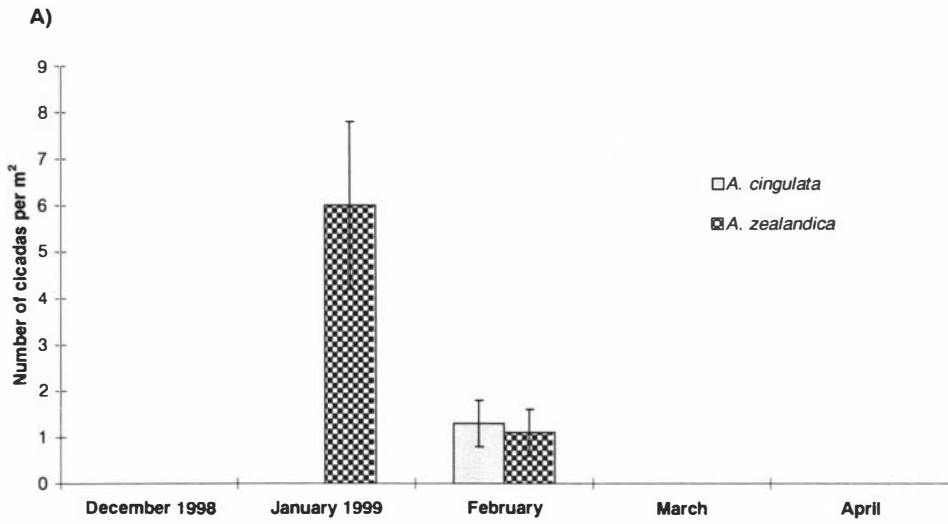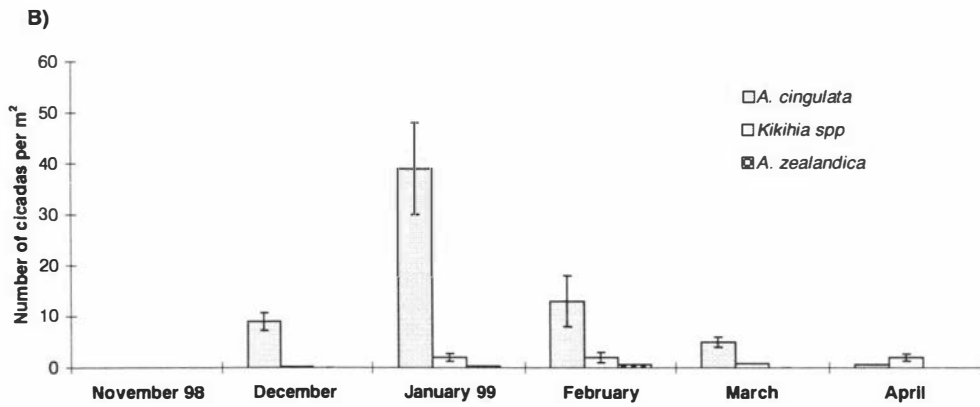
Figure 2.2. A) Density of *A. cingulata* and *A. zelandica* emerging each month from 30 Fixed Emergence Traps at Eastwoodhill Arboretum (n= 99, total area sampled 12 m²). B) Density of *A. cingulata* , *Kikihia* spp., and *A. zelandica* emerging each month from 16 Fixed Emergence Traps on Mokoia island (n = 489, total area sampled 6.4 m²). Bars indicate standard error.

The exoskeleton persistence score from the Mokoia fixed sampling area was 7%. After subtracting 7% of exoskeletons from each quadrat I was left with 1179 exoskeletons. (Fig. 2.3b). The species represented and their proportions were, *A. zelandica* (58%), *A. cingulata* (40%), and *Kikihia* spp (2%). Of these, 696/1179 exoskeletons were from male cicadas (59%) and 483/1179 were from female cicadas (41%). Counts per quadrat ranged from zero to 48 exoskeletons, giving a combined mean density of 12 cicada exoskeletons per square metre on Mokoia (SE= 2). Like EWH, the highest exoskeleton counts on Mokoia were recorded in the first two months following the conclusion of the emergence period. By July 1999 exoskeleton counts had decreased by about two fold.

Exoskeleton counts were highly variable at EWH, the range was almost twice as high as that recorded on Mokoia. 55% of EWH quadrats gave a zero exoskeleton count. On Mokoia the range was small, and only 26% of quadrats gave a zero exoskeleton count. This indicates a higher degree of patchiness in EWH cicadas compared to Mokoia. Additionally, *A. zelandica* was the most common species recorded from the random exoskeleton counts on Mokoia. This is in contrast to the results from the fixed sampling area and emergence traps set up at the bush patch on Mokoia. The Mokoia random exoskeleton counts and the nymph samples were conducted all over the island away from the bush patch. The bush patch (called Isabel`s Camp) is obviously an *A. cingulata* strong hold, with very few *A. zelandica* (1%) showing up in either the fixed sampling area or the emergence traps. This is interesting because it may imply that mixed broods of *A. zelandica* and *A. cingulata*, while not uncommon, are best avoided by both species when possible. The bush patch differs little in regards to the composition and age its plant species to suggest host plant preferences as an explanation. The random exoskeleton counts indicate that *A. zelandica* is the most abundant cicada species at EWH and Mokoia.

D) Nymph sampling

All nymph samples were grouped according to instar age, no attempt was made to identify the species of any nymphs collected. Nymph sampling was conducted between the months April to August 1999. Except for August, the 20 sample holes dug each month were taken from the centre of each exoskeleton quadrat. It was assumed that species compositions above ground would reflect the species compositions below ground. Therefore, the species compositions from the random exoskeleton counts are
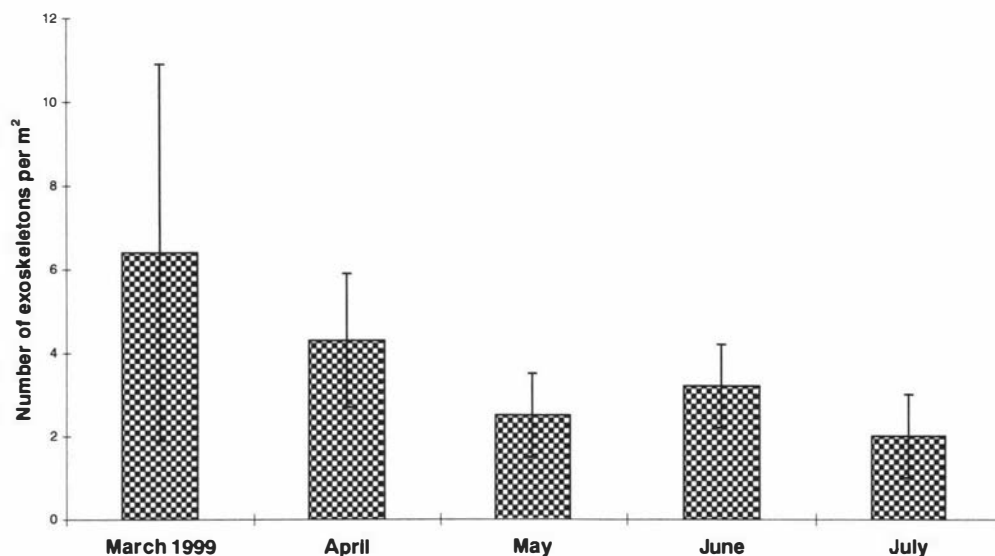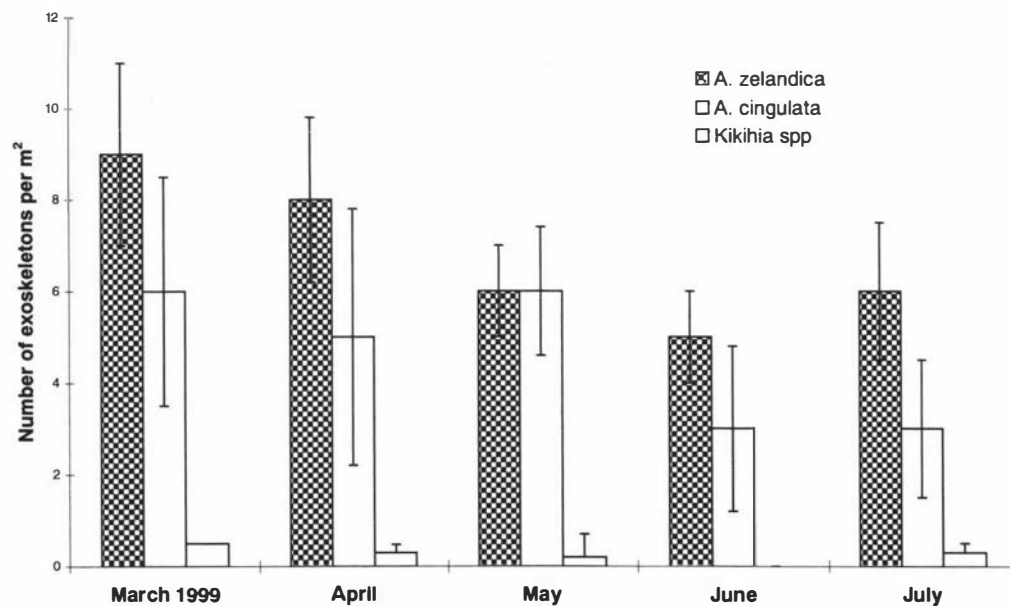
Figure 2.3. A) Monthly densities of *A. zelandica* exoskeletons at Eastwoodhill Arboretum (n= 369, 100 random sample points, total area sampled 100 m²). B) Monthly densities of cicada exoskeletons on Mokoia Island (n= 1179, area sampled 100 m²). Bars indicate standard error.

used to calculate the proportional densities of 5th instar cicadas for each represented species.

I dug in total 100 holes, to a depth (when possible) of 70 cm at both study sites. At EWH I collected a total of 114 nymphs from a surface area of 3.24 m^2 (Fig. 2.4a). The number of nymphs per hole ranged from zero to 5 per hole, with a combined instar mean density of 35 nymphs per square metre (SE= 5). I found that 77/114 nymphs were in their 5th and last instar (66%). The rest of the nymphs were from their 4th instar (30/114, 26%) and 3rd instar (3/114, 8%), nymphs in their 2nd instar were found at EWH. This gave a mean 5th instar density of 24 nymphs per square metre (SE= 5). Although the monthly standard errors were high at EWH, there was on average a three-fold increase in the density of 5th instar nymphs over winter.

On Mokoia I randomly collected a total of 702 nymphs from the same surface area sampled at EWH (Fig. 2.4b). The number of nymphs per hole ranged from zero to 26 per hole, with a combined instar mean density of 217 nymphs per square metre (SE= 23). I found that 320/702 nymphs were in their 5th instar (46%), 253/702 were in their 4th instar (36%), 102/702 were in their 3rd instar (15%), and that 27/702 were in their 2nd instar (3%). This gave the 5th instar nymphs a mean density of 99 per square metre on Mokoia (SE= 10.5). Like the EWH data, the density of 5th instar nymphs on Mokoia increased over winter. On Mokoia there was also a three-fold increase in the density of 5th instar nymphs between the months April to August 1999.

Overall, I found Mokoia had a combined nymphal density about six times greater than that found at EWH. The EWH nymphal population shows a higher degree of patchiness and is composed mostly of 5th instar nymphs. On Mokoia the situation is different, 5th instar nymphs do not dominate the nymphal population, and there is a greater proportion of 4th and 3rd instar nymphs. This implies that on Mokoia nymph cohorts from different age broods are practically living on top of one another, while at EWH single age broods are more distantly spaced.

2) *Mycoses in cicada adults and nymphs*

Results from the fungal screening program conducted at both study sites are split into two groups. The first group is genera of fungi extracted from the adult cicadas, and the

nymphal densities for instars 2- 5 at Eastwoodhill Arboretum

A)

nymphal densities for instars 2- 5 on Mokoia Island

B)
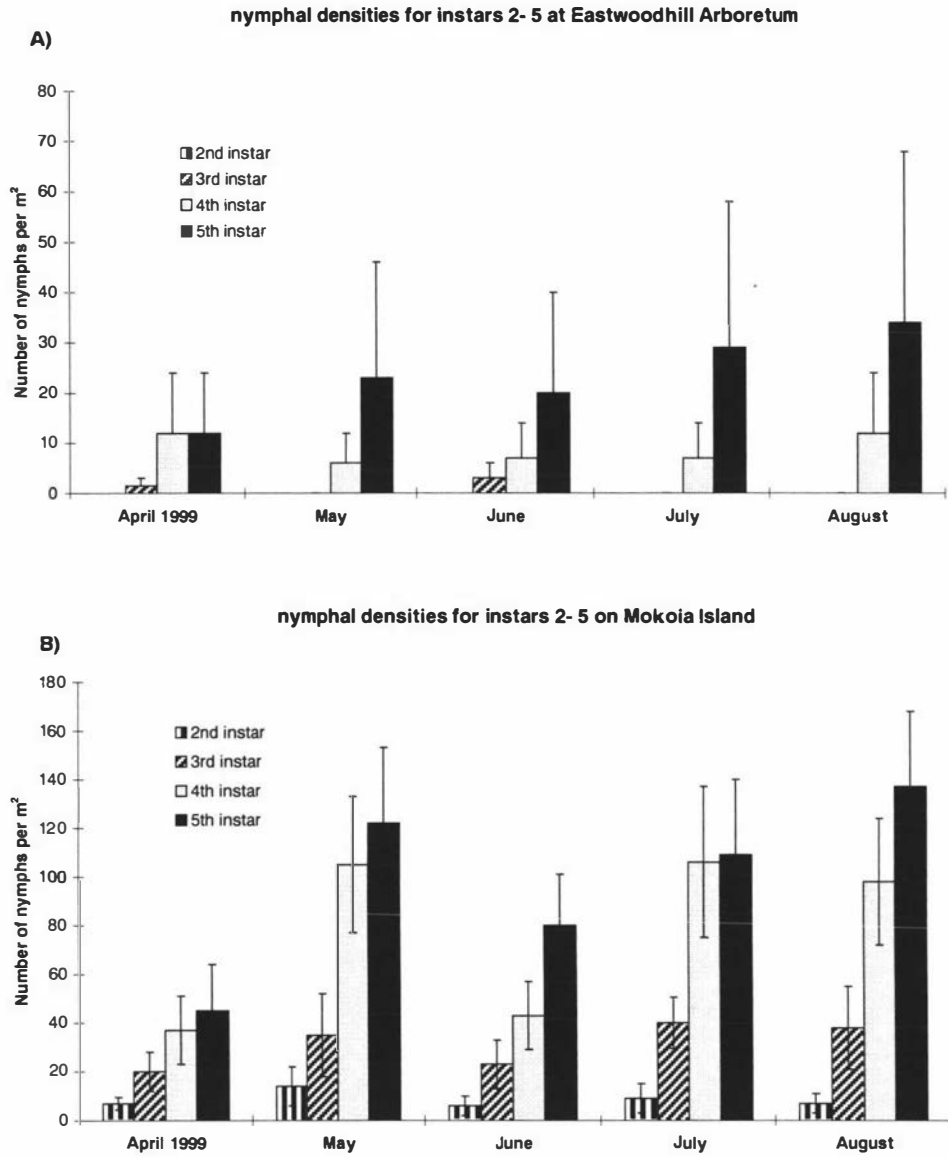
Figure 2.4. A) Combined cicada species nymphal densities for instars 2- 5 at Eastwoodhill Arboretum (n= 113, 100 random holes, total area sampled 3.24 m²). B) Combined cicada species nymphal densities for instars 2- 5 on Mokoia Island (n= 702, 100 random holes, total area sampled 3.24 m²). Bars indicate standard error.

second group is genera of fungi extracted from cicada nymphs. The adult sample group are further split into three subgroups, a) cicadas caught alive on vegetation, b) cicadas found dead on ground, and c) cicadas found dead attached to vegetation (Table 2.2). Only on Mokoia were dead cicadas found attached to the vegetation, at EWH all dead cicadas were found on the ground.

A) Cicadas caught alive on vegetation

At EWH and Mokoia 30 adult cicadas were non-randomly collected from several transect lines. This involved collecting male and female cicadas at 20 m intervals along bush edges and tracks at both study sites. Once I had collected one male and one female from each sample point I moved the 20m to the next sample point. If no cicada was caught within 10 minutes I would abandon the search and move to the next sample point. None of the cicadas collected had any obvious injuries.

During February 1999 I collected 15 male and female cicadas from 27 sample points at EWH, and the same number of cicadas from 17 sample points on Mokoia. From this, I extracted fungi from 2 adult female *A. zelandica* from EWH (Fig. 2.5a), and 5 adult cicadas from Mokoia (2 female *A. zelandica*, 1 male *A. zelandica*, and 2 male *A. Cingulata,* Fig. 2.5b). This gave an infection rate of 7% in the live adult population at EWH, and a 17% infection rate on Mokoia (Table 2.1).

At least two genera of fungi were extracted and identified from the EWH cicada samples. These were *Aspergillus* and *Penicillium*, also found were various spore types of *Fusarium. Aspergillus* was found on one cicada, and *Penicillium* was found on the another, *Fusarium* spores were found in both samples. All three fungi are opportunist insect pathogens, and probably indicate the cicadas were already sick prior to infection.

On Mokoia fungi was extracted from 5 cicadas. These were mostly *Conidiobolus*, *Aspergillus, Penicillium*, and *Mucor*. Other fungal spores were also found, but these were mostly *Fusarium* and *Alternaria. Conidiobolus* is the only primary entomophagous pathogen found, the other genera, including *Mucor* are common insect pathogens and saprophytes, but usually occur in sick or dead insects as secondary infections.
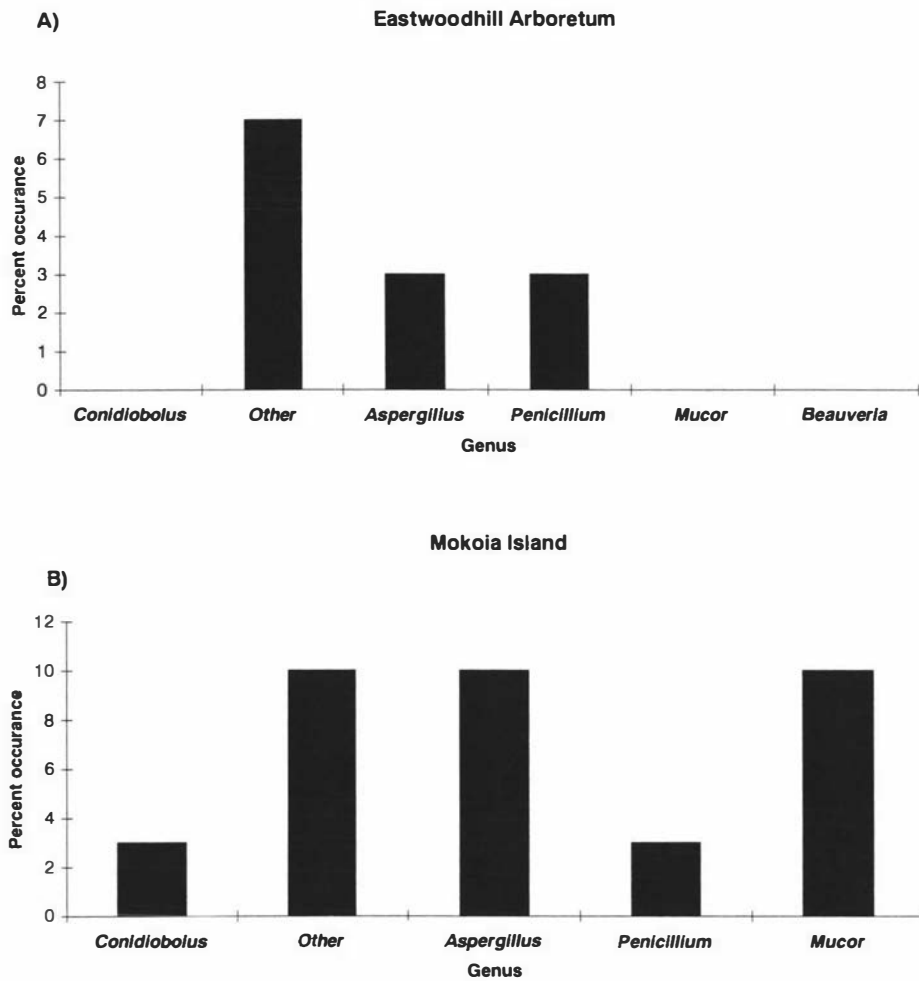
Figure 2.5. A) Proportion of the cicada sample population infected with fungi at time of death from Eastwoodhill Arboretum (n= 30). B) Proportion of the cicada sample population infected with fungi at time of death on Mokoia island (n= 30). From cicadas caught alive on vegetation vegetation.

B) Cicadas found dead on ground

Dead cicadas were easily found during late February 1999 at both study sites. At EWH I collected the 15 male and female cicadas found dead on the ground while checking the fixed sampling areas and emergence traps. From these samples I extracted fungi from 7 cicadas (5 male *A. zelandica* and 2 female *A. cingulata*). This gave an infection rate of 23% for cicadas sampled at EWH. On Mokoia fungi was extracted from 21 cicadas (12 male *A. cingulata*, 2 male *A. zelandica*, 5 female *A. zelandica*, and two female *A. cingulata*). This gave an occurrence rate of 70% for cicadas found dead on the ground on Mokoia.

Four genera of fungi were extracted from the seven infected cicadas found at EWH (Fig. 2.6a). These were, *Aspergillus, Penicillium, Mucor*, and *Beauveria*. *Mucor* was the most common genus found, turning up in 5/7 of infected cicadas. *Beauveria* and *Penicillium* were found on two cicadas each, and *Aspergillus* was found on one cicada. *Beauveria* is a common entomophagous pathogen, with a wide host range, however its presence does not imply cause of death. *Mucor* is common soil fungus and a vigorous saprophyte which usually occurs *post mortem*.

On Mokoia at least four genera of fungi were extracted from 30 cicadas found dead on the ground (Fig. 2.6b). The genera of fungi found were, *Conidiobolus, Aspergillus, Penicillium, Mucor*, and other (*Fusarium* and *Alternaria*). Like EWH the most common fungus found was *Mucor* (11/21). *Aspergillus* and *Penicillium* were both found on 3 cicadas, and *Conidiobolus* was found on one cicada. The cicada infected with *Conidiobolus* was the only cicada exhibiting mycosis during the time of collection; the other samples either had moulds not yet visible to the naked eye, or developed moulds *post mortem* during incubation. Like EWH most fungi extracted were opportunist pathogens or saprophytes (20/21).

C) Cicadas found dead attached to vegetation

While over 100 dead cicadas were found dead attached to vegetation on Mokoia, at EWH all dead cicadas were found on the ground. The first 31 cicada samples had their fungi extracted and identified (Fig. 2.7). Only 4/31 cicadas found in this category were not exhibiting mycosis during the time of collection. All the other cicada samples were firmly attached to the vegetation by hyphae (27/31), and had their rostrum inserted as if
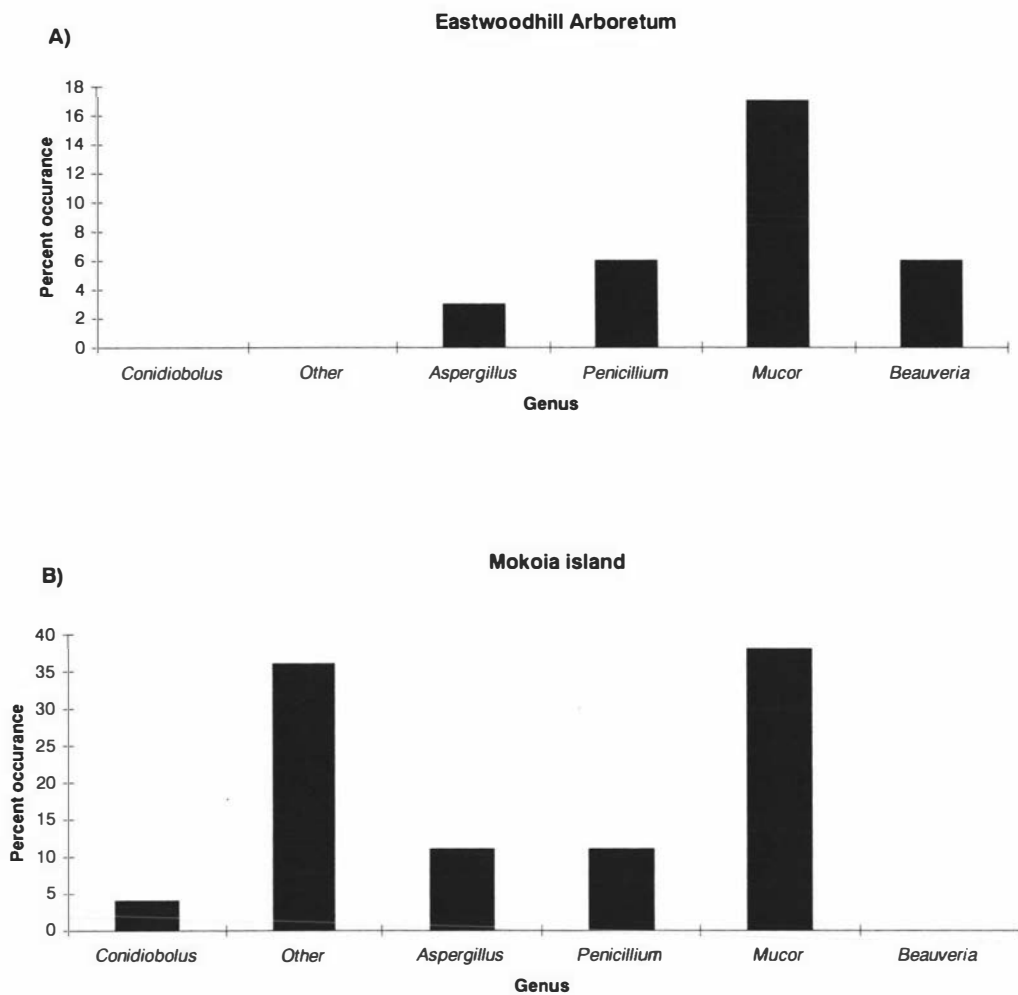
Figure 2.6. A) Genera of fungi cultured from cicadas found dead on the ground at Eastwoodhill Arboretum (n= 30 cicada samples). B) Genera of fungi cultured from cicadas found dead on the ground on Mokoia island (n= 30 cicada samples).

feeding (25/31). Two female *A. zelandica* had their ovipositors and rostrums inserted. There was no bias towards either sex with 54% cicadas were male, and 46% female. In this category *Conidiobolus* was the most common fungus genera found (15/31, P < 0.05). Its likely *Condiobolus* was on all cicadas in this category, however, *Aspergillus* had spread over samples within days of collection. *Aspergillus* was originally found on 6/31 cicada samples, and *Mucor* and *Penicillium* were found on 3 cicadas each. Even though cicadas were enclosed in sterile containers in a fridge at 4°C, *Aspergillus* completely displaced *Conidiobolus* within one week.

It seems likely *Conidiobolus* was the fungus responsible for attaching cicadas to the vegetation. Cicadas were collected from a wide range of trees and shrubs. Cicadas that were alive seemed lethargic and unwilling to fly away, even when handled. It seems the cicada dies within hours, upon death, hyphae grow out from between the legs and firmly attach the dead cicada to the trunk, branch, or twig. *Condiobolus* is a common pathogen with a wide host range, however, no other insects were found attached to the vegetation in the same manner as the cicadas on Mokoia. I was not able to culture this fungus. Identification to species will be done when fresh samples are collected.

D) Nymph samples

Very few nymphs from either study site had moulds visible to the naked eye. Only nymphs in their 5th instar had infections at time of collection. Therefore, I did not include cicadas from earlier instars in the infection rate analysis. Because all nymphs were in the soil surrounded by a multitude of fungal pathogens and saprophytes I did not incubate any samples. All recorded fungi genera come from nymphs exhibiting mycosis at time of death. None of the nymph samples had milky haemolymph or injuries.

At EWH 77 fifth instar nymphs were recovered, from these I extracted fungi from two nymphs. Both nymphs were infected with fungi from the genera *Beauveria*. This gave a nymph infection of 3% at EWH. On Mokoia 320 fifth instar nymphs were collected, from these I extracted fungi from 3 nymphs. All nymphs were infected with *Aspergillus* and *Mucor*. This gave a nymph infection rate of 1% on Mokoia.

Beauveria is a common soil pathogen, so it is surprising it was not found on Mokoia. It is possible I missed *Beauveria* on Mokoia, or that *Aspergillus* and *Mucor* obscured its
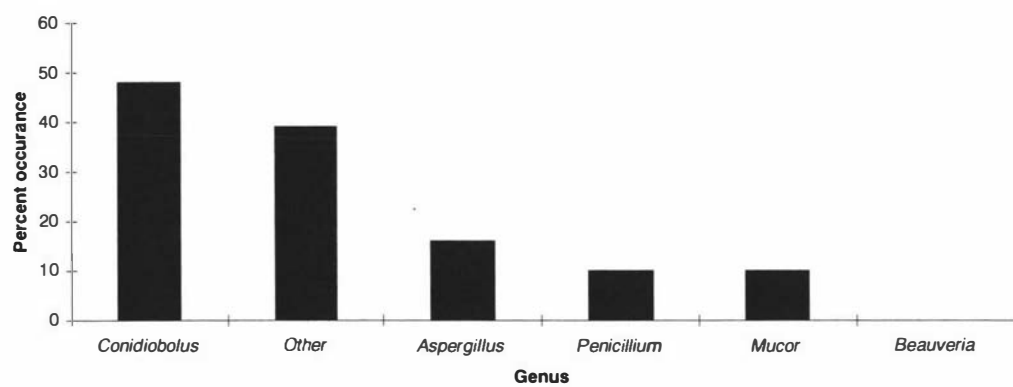
Figure 2.7. Percent occurence of fungi associated with dead cicadas on Mokoia Island. From mature cicadas found dead attached to vegetation (n= 31 cicadas).

presence in the samples. Whiles it is likely that *Beauveria* caused the death of the EWH nymphs, it is unlikely the same can be said for the fungi found on the nymphs on Mokoia. While the sample size it small, it seems the EWH nymphal population has a higher incidence of disease compared to Mokoia.

During field trips to Mokoia Island in May 2000 several cicada nymphs from the genera *Amphipsalta* and *Kikihia* were found with moulds identified as *Paecilomyces cicadae*. This disease had been originally overlooked in the Mokoia cicada population, and not identified at EWH.

Discussion

In addition to the investigation into mycoses in cicadas, this study compared three independent census methods replicated at two study sites to estimate the mean density of emerging cicadas per unit area over time. The density and age distribution of the nymphal population at each study site was estimated using independent census methods.

I have shown that the cicada population suddenly rose from zero to a high level within a narrow time transition at both study sites (10-14 days). I also demonstrated that the emerging cicada sex ratio is often biased towards males ($P < 0.05$), and that great variability exists between the census methods used to estimate emergent cicada densities. The resulting independent density estimates for emerging cicadas ranged from 4 to 10 cicadas per square metre at EWH, and 12 to 93 emerging cicadas per square metre on Mokoia. Nymphal densities ranged from 30 to 35 nymphs per square metre at EWH, and 194 to 240 nymphs per square metre on Mokoia. Fifth instar nymph densities ranged from 19 to 29 nymphs per metre square at EWH, and 89 to 110 nymphs per square metre on Mokoia.

At both study sites the fixed sampling areas gave the highest emerging cicada density estimates. This is not surprising as emergent 5th instar nymphs will aggregate on tree trunks and other vertical surfaces to shed their last exoskeleton, and all fixed sampling sites were situated around one or more tree trunks. This was done to investigate

exoskeleton persistence on the ground and vertical surfaces. On Mokoia the emerging cicada density was 9.3 times greater compared to EWH.

The resulting cicada densities from the emergence traps gave the next highest estimates at both study sites. There was great variability between trap counts at both study sites, with many traps remaining empty throughout the emergence period (e.g., EWH= 55% zero counts, Mokoia = 26% zero counts). It is likely that traps more accurately measure the actual emerging cicada densities compared to the fixed sampling sites. This is because counts from the traps cannot be confounded by cicadas entering the sample area from outside the trap.

At EWH cicada broods were so widely spaced that too few traps were set up at the study site to accommodate for this. If I plot the frequency distribution of the number of cicadas found in the emergence traps at both study sites I find they are far from normally distributed (Fig. 2.8a,b). At EWH the frequency distribution is skewed to the left indicating a hight frequency of low counts, while the tail indicates a low frequency of high counts (Fig. 2.5a). If the frequency of high counts are underrepresented there is a real possibility of underestimating the population size. This may indicate that too few traps were used at EWH. On Mokoia the frequency distribution of cicadas found in the emergence traps shows an even distribution of cicada counts (Fig. 2.5b). This indicates that cicada broods on Mokoia were more common with less clumping compared to EWH.

Random exoskeleton counts gave the lowest emerging cicada densities at both study sites. This is probably a result of starting the exoskeleton counts two months after the start of the emergence period. Therefore, it is possible many exoskeletons had already been removed or degraded prior to the sampling regime.

Fifth instar nymph mean densities were higher than recorded emerging nymph densities at both study sites. Additionally, the age distribution of both nymphal populations is skewed towards the 5th instar stage. This population bottleneck has been reported to be caused by nymphs remaining in their 5th instar for more than one year. This variable development has been demonstrated in *Kikihia muta* nymphs (Cumber 1952). It is possible that this is a kind of out breeding mechanism that enables nymphs from

Emergence traps frequency distributions at Eastwoodhill Arboretum

Emergence traps frequency distributions on Mokoia Island
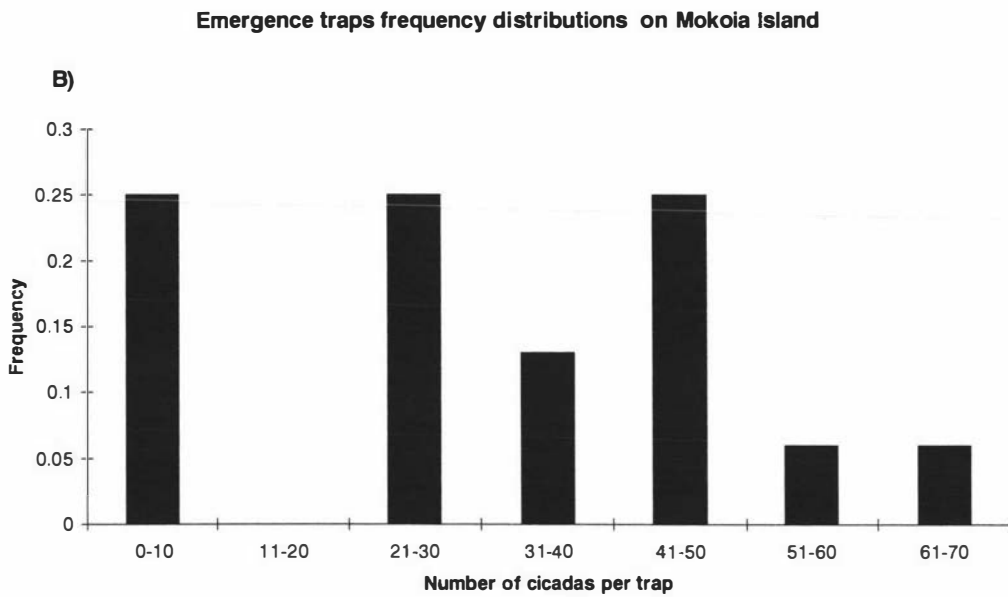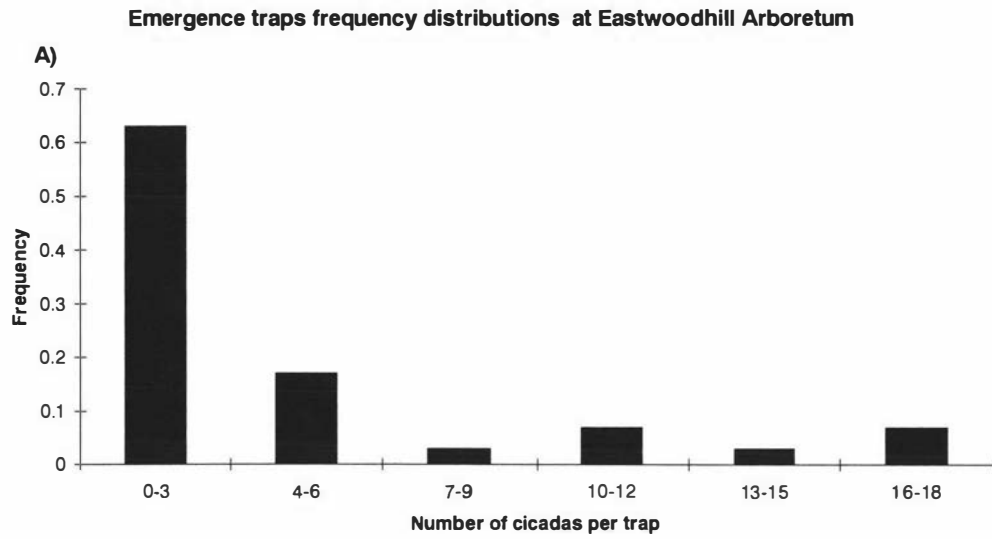
Figure 2.8. A) Frequency distribution of the number of cicadas found in the emergence traps at Eastwoodhill Arboretum (n= 99, 30 X 0.4 m² traps). B) Frequency distribution of the number of cicadas found in the emergence traps on Mokoia Island (n= 489, 16 X 0.4 m² traps).

different age broods to emerge and mate together in any one year (Marshall and Cooley 2000). Another explanation is that cicadas during the early instar stages suffer high mortality rates, while the 5th instar nymphs have high survivorship.

Cumber (1952) ascertained that *K. muta* nymphs take one year to reach their 3rd instar from oviposition. Fourth instar nymphs take another two years to reach the 5th instar stage, and the fifth instar stage may last another year or more. Therefore, the adult emergence from any one years oviposition may thus occupy two adjacent years. Moulds (1990) comments on a nine-month juvenile period in two non-periodical grass species, *Parnkalla muelleri* and *Cicadetta crucifera*. Beamer (1928) reported a four-year juvenile phase in *Melampsalta calliope* in Kansas. In New Zealand, Cumber (1952) reported three or four years in *Kikihia muta,* and there is a seven-year juvenile phase in the Japanese nonperiodical cicadas *Graptopsaltria nigrofuscata* and *Oncotympana maculaticollis* (Kato 1956). As mentioned, very little information is available on the cicada nymphal stage, and at present no mechanism exists to explain variable development in nonperiodical cicadas.

The cicada population density on Mokoia was six to nine times greater than the EWH population. Several factors may have been responsible but none can be proven with our data. There is a possibility that the cicada population at EWH, while not itself randomly distributed, may represent an aggregate of local populations of differing densities. Since EWH was populated largely by *A. zelandica* and Mokoia was dominated by *A. cingulata* and *A. zelandica*, the different densities might simply reflect differences in productive rates of the two populations. In view of the close relationship of the two species in these and in other respects, it would seem unlikely that there would be differences in fecundity of such a magnitude as to account for the great disparity in numbers between the two populations.

One possibility is that there is more food available on Mokoia compared to EWH. *Amphisalta* nymphs feed on sap from roots of woody plants, but there is no evidence that they have host specificities or preferences among the different species of trees and shrubs that might constrain their distributions underground. While it seems unlikely that food availability in general could account for the observed differences in population densities between the two study sites, the differences in the distribution of their host

plants could. The EWH cicada population may have a more patchy distribution because their host plants are clumped to a greater degree than found on Mokoia. Mokoia is almost entirely covered in forest and nymphs may be more evenly distributed because their egg nests are distributed over a wider area reducing aggregation below ground.

Another possibility is differential mortality of the nymphs in the two environments in their first and 5th instar. Newly hatched and 5th instar nymphs must work their way through layers of leaf litter before they can achieve their next stage in their development. The leaf litter contains a great number of predatory arthropods, and nymphs must run this gauntlet before proceeding with their development (Kalisz 1994). Therefore, nymphs in environments with a well-developed leaf litter may suffer greater predation pressure compared to environments with little or no leaf litter. However, this as an explanation seems unlikely as the forest floor litter, with its rich biota, is much less developed at EWH compared to Mokoia.

The highly clumped cicada population at EWH indicates some external factors and/or interaction among cicada nymphs and/or adults are affecting their distribution. The possibility exists that the adult cicada population at EWH is not entirely composed of cicadas that emerged within the arboretum. The proportion of migrant cicadas mixing with the emergent cicada population requires further investigation before a reliable adult population census can be made from nymphal density estimations. This is not a problem on Mokoia Island as cicadas are unlikely to be able to fly the 2.1 km across the lake to Mokoia from the nearest mainland.

I isolated several genera of fungi from the adult cicada and nymph samples at both study sites. The frequency of infection varied between categories and study sites. The study sites differed little in types of soil saprophytes and/or faculative insect pathogens extracted from cicadas, however, they did differ in the genera of entomophagous fungi (obligate insect pathogens). At EWH I found one genus that can be considered an obligate insect pathogen, and that was *Beauveria*. On Mokoia the dominant mycopathogen found was *Conidiobolus*. *Mucor, Aspergillus* and *Penicillium* were the most common faculative fungal pathogens at both study sites. *Mucor* was most commonly found on dead cicadas on the ground. *Aspergillus* and *Penicillium* infection frequencies were higher in adult and nymph samples on Mokoia.

At both study sites the nymphal populations recorded the lowest proportion of individuals infected. At EWH only fungi from the genus *Beauveria* were extracted from dead nymphs. On Mokoia no fungal parasites were extracted from nymphs, only common soil saprophytes. Adult cicadas exhibiting mycosis were more commonly found on Mokoia, especially during February.

The differences in obligate insect pathogens between the two sites is likely due to differences in their soil moisture levels. EWH is mostly mature trees planted on pasture. In contrast, Mokoia is mostly forested and the soil is covered with damp leaf litter. Entomopthorales such as *Cordyceps* and *Conidiobolus* spp are more commonly reported infecting Lepidoptera from undisturbed habitats, while Deuteromcete fungi such as *Beauveria* are more commonly found infecting soil insects in pasture and orchard habitats (Baker and Baker 1998). Wicklow (1973) found that decreases in leaf litter and nitrogen by approximately 50% were accompanied by reductions of fungal propagules of up to 80%. Wicklow (1973) reported that the effect of removing the leaf litter system created conditions favouring the development of fungal species capable of growth at elevated soil temperatures and/or able to survive periods of excessive drought, such as *Aspergillus fumigatus*.

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Chapter 3

Seasonal and habitat induced changes in *Aspergillus fumigatus* densities in surface soils on Mokoia Island

Introduction

This chapter outlines experiments to test whether leaf litter removal favours the establishment of the fungus *Aspergillus fumigatus* in the surface soil. The primary aim of this experiment is to compare seasonal changes in *A. fumigatus* in disturbed versas non-disturbed sampling plots on Mokoia Island (Lake Rotorua).

Aspergillus is a Deuteromycete, filamentous, cosmopolitan fungus commonly isolated from soil, plant debris, and air. The genera *Aspergillus* and *Penicillium* together constitute the most ubiquitously distributed moulds in the world (Atlas and Bartha 1998). *Aspergillus* includes over 185 species, and over 20 species have been reported as causative agents of opportunistic diseases in man, other mammals, birds, arthropods, and marine life (Adriole 1993). Among these, *A. fumigatus* is the most commonly isolated species, followed by *A. flavus* and *A. niger*. In addition, diseases of gorgonian or soft corals caused by *A. sydowii* are increasing, resulting in mass mortalities of extensive stands of seas fans throughout the Caribbean (Smith *et al.* 1996; Goreau *et al.* 1997). *Aspergillus* spores are very small (2-3 micrometers diameter), and can be carried on dust particles. Studies have demonstrated that *Aspergillus* spores are being carried by global air currents from northern Africa across the Atlantic to the Caribbean (e.g., Smith *et al.* 1996; Cervino *et al.* 1997). High levels of *A. fumigatus* coupled with dry dusty conditions also pose a serious human health risk (Lecours *et al.* 1986). *Aspergillus fumigatus* is one of the most commonly reported avian disease causing agents in the world (Adriole 1993; Bauck 1994), and aspergillosis is incurable in wild bird populations. In New Zealand, aspergillosis has been identified as a major mortality

factor affecting free-living Hihi or stitchbird, *Notiomystis cincta,* on Mokoia Island (Alley *et al.* 1999).

The importance of *A. fumigatus* as a disease organism means that it is important to understand the conditions leading to its prevalence. Wicklow (1973) conducted leaf-litter removal experiments on microfungal populations in surface soils of manipulated prairie stands in Wisconsin, USA. Wicklow found that the effect of removing all ground cover created conditions favouring the development of *A. fumigatus*. Wicklow demonstrated that by removing all leaf litter in sample plots, conditions favoured the establishment of fungal species capable of growth at elevated soil temperatures and/or able to survive periods of excessive drought through the production of resistant propagules, i.e., sclerotia in the case of *A. fumigatus*. *Aspergillus fumigatus* is known to grow well at 46^0C and higher. It has been frequently isolated from dry soil in high densities, particularly at exposed sites such as cultivated and desert soils (Moubasher and Abdel-Hafez 1978; Ali *et al.* 1975), disturbed sites associated with logging activity (Akpata and Ekundaya 1983), dry prairies (Orpurt and Curtis 1957), alluvial grasslands (Apinis 1963), open savannah (Eicker 1974), and sand bars (Gochenaur and Backus 1967).

In temperate climates like New Zealand, water activity is most likely to be a key factor influencing microorganisms like *Aspergillus* that live in the soil surface layer or on exposed surfaces. For species like *A. fumigatus,* surface soil temperatures rarely exceed their thermotolerance limit (46-50^0C). For most aerobic soil microorganisms, approximate water activities above 0.96 (or 96% RH) are required for optimum growth (Atlas and Bartha 1998). *Aspergillus* fungi are among a small group of fungal species capable of growth at low soil water activities of around 0.75 (Smith and Hill 1982, Brock *et al.* 1984). This means *Aspergillus* species are not only capable of survival in dry environments, but also able to survive in media with elevated solute concentrations, such as salty water.

Aspergillus fumigatus is a noxious cosmopolitan species and has many weed-like characteristics. For instance, the species can spread very rapidly and produce huge quantities of spores. *Aspergillus* spores are primarily wind dispersed, and the fungus can spread from both small hyphal fragments and from spores. *Aspergillus fumigatus*

produce toxic chemicals such as aflatoxin, that suppress other organisms. Most weedy species have global distributions, and compete best in disturbed environments (Lugo 1988). Because of this, they are most commonly identified associated with exposed habitats such as forest edge zones. Many groups of forest soil fungi are likely to be affected by habitat change causing increased exposure to climatic factors, which raises soil temperatures and decreases moisture levels. In cleared areas, daytime temperatures are generally higher and night temperatures generally lower than in naturally vegetated areas. This leads to greater temperature changes both at the surface and in the upper layers of the soil, and an increased incidence of frost (Geiger 1965). Air temperatures at the edge of a forest remnant can be significantly higher than those found in either the interior of the remnant or the surrounding cleared/agricultural land (Geiger 1965; Kapos 1989). Therefore, deforestation retards soil production processes, exposes the forest floor to desiccation and erosion, and dry winds can carry dust and spores high into the atmosphere.

This chapter outlines experiments conducted on Mokoia Island to investigate seasonal variations in *A. fumigatus* in the surface soil and air. By identifying seasonal variations in *A. fumigatus* colony counts, I aim to identify the most likely periods of disease prevalence and transmission on Mokoia. Additionally, leaf litter removal experiments were conducted to investigate whether habitat disturbance is a factor influencing the abundance of *A. fumigatus* in forest habitats. At the start of these experiments I created the opportunity for *A. fumigatus* to invade the forest surface soil by exposing the surface soil layer to the air over a 12-month period at 0.4 m X 10 treatment plots.

This chapter addresses the following questions, 1) Do *A. fumigatus* densities in the surface soils on Mokoia vary seasonally? 2) Do *A. fumigatus* densities vary significantly between sample plots? 3) To what degree does leaf litter removal affect the density of *A. fumigatus* in the surface soil?

Study Site

Mokoia Island is in Lake Rotorua, in the North Island (38°06′S, 174°55′E). With an area of 135 ha, it is largest inland island in New Zealand (Figure 3.1). The shortest
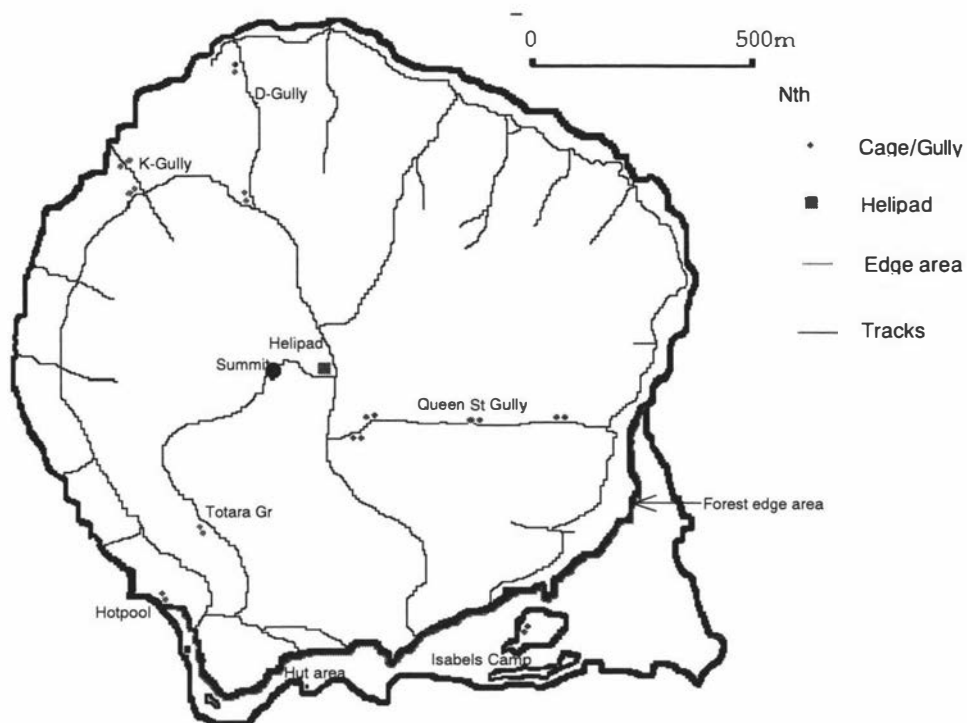
Figure 3.1. Map of Mokoia Island showing the locations of the sample
sites used in this study

distance from the mainland is about 2.1 km. It has secondary forest, which has been regenerating for about 50 years. In general, the soil of Mokoia is sandy (volcanic-pumice), slightly alkaline (pH = 6.5- 8), with high organic content and low clay content.

Mokoia has a long history of Maori occupation, mainly by Te Arawa. Crops grew well in the fertile soil for hundreds of years, and most of the island was fired, cleared and terraced (Andrews 1992). Mokoia was also modified by deliberate introduction of many native trees such as karaka (*Cordynocarpus laevigatus*), whau (*Entelea arborescens*), totara (*Podocarpus totara*), puriri (*Vitex lucens*), kowhai (*Sophora spp.*) and southern beech (*Nothofagus spp.)*. Europeans arrived on Mokoia not long after the 1823 Nga Puhi invasion. European missionaries planted exotic tree species like pine tree (*Pinex radiata)*, poplar (*Aspen spp.*) and a variety of fruit trees, and introduced cattle (*Bos taurus*), goats (*Capra hircus*), sheep (*Ovis aries*), horses (*Equus callallus*), pigs (*Sus scrofa*), and cats (*Felis catus*). Norway rats (*Rattus norvegicus*) were introduced accidentally, and were abundant on Mokoia by 1840 (King 1984). There are no reports of possums (*Trichosurus vulpecula*) or mustelids ever being present on Mokoia.

Cultivation had stopped on Mokoia by about 1950, and the island was made a Wildlife Refuge under the Wildlife Act (1953). Pheasants were farmed as a commercial venture from 1952-56, and grain fed to pheasants probably encouraged irruptions of the rat and mouse (*Mus musculus*) populations (Beveridge and Daniel 1965). Weka (*Gallirallus australis*) were translocated to Mokoia sometime in the 1950s, and are still there. The first restoration attempts, in the mid 1960s, involved planting thousands of native tree and fern saplings. Most of these were probably eaten by the abundant rats, and the planting had negligible effect on the present vegetation (Wallace 1993).

Goats were introduced in 1985 to control the blackberry (*Rubus fruticosus)* on the eastern flat, which was fenced off. However, the goats escaped into the bush and began browsing the regenerating forest. By 1989 the understorey was open with no further regeneration of canopy species taking place. In 1989-90 the Department Of Conservation (DOC) successfully eradicated rats and goats from Mokoia.

Since the rats and goats were eradicated, toutouwai (North Island robin, *Petroica australis*), tieke (saddleback, *Philesturnus carunculatus*), and hihi have all been

reintroduced to Mokoia. There are several other bird species, which either survived human occupation or naturally recolonised the island. Tui are the only other honeyeater species, and are found at high densities. Lizards were thought to be absent, but speckled skinks (*Oligosoma infrapunctatum*) were discovered on the island in 1993. A mouse eradication was attempted in September 1996, but was unsuccessful. Another mouse eradication was attempted in September 2001, as this thesis was being written.

The island's vegetation is now composed largely of understorey species such as five-finger (*Pseudopanax arboreus*), kawakawa (*Macropiper excelsum*), mahoe (*Melicytus ramiflorus*) and rangiora (*Brachyglottis repanda*). The vegetation is low and scrubby on the ridges, particularly near the summit, whereas there is a closed canopy and open forest floor in gullies and near the lakeshore. The canopy species are largest in gullies and bush edges, where mahoe, kohuhu (*Pittosporum tenuifolium*) and treefern (*Cyathea spp.*) dominate. On the south facing-slopes mamaku (*Cyathea medullaris*) is the dominant canopy species. Amongst the fragments of bracken (*Pteridium aquilinum*) and blackberry on the north-facing slopes the dominant species are cabbage tree (*Cordyline australis*) and five-finger (Beadle and Ecroyd 1990; Perrott and Armstrong 2000).

Methods

The leaf litter removal experiments were started on Mokoia Island during May 2000 and concluded during April 2001. Ten experimental sites were established at recent hihi nesting locations on Mokoia Island. Eight of the sites were in gullies, two others being near the forest edge. All sample sites were situated under canopy cover in areas with a permanent covering of leaf litter.

At each site I set up two paired mesh-covered triangular cages, each with a surface area of 0.4 m². I removed all surface debris (treatment cages) exposing the surface soil layer in one cage per site. Surface debris were left undisturbed (control cage) in the other cage. Leaf litter removal was performed once at the start of the experiment at each site. Twelve months prior the start of the leaf litter removal experiments 10 other treatment cages were established in a small forest patch (140 m², Isabel's Camp) in the middle of the 'old horse paddock' on the eastern flat. This was done to investigate the persistence of *A. fumigatus* in the surface soil two years following the leaf litter removal event. In

total there were 30 cages in the experiment; 10 untreated (control cages), 10 treatment cages, and 10 treatment cages more than one year old.

During the placement of each cage, and thereafter on a monthly basis, soil samples (5 X 10 g) were removed by scooping the surface soil layer to a depth of 2 cm with a sterile container. The soil samples were then transported back to the Plant Pathology Laboratory at Massey University and stored at 3 ^{0}C until processing (usually within 2 to 4 days of collection).

In the laboratory, soil samples were crushed by careful hand manipulation for determinations of moisture content. Moisture content was calculated to estimate actual dry soil weights from wet soil samples. Moisture content for each soil sample was estimated by removing 1g of wet soil and drying this in an oven at 37^{0}C until no further weight loss occurred (2 to 3 days). The percent soil moisture was calculated as 1 - dry weight X 100.

Numbers of *A. fumigatus* propagules per gram of dry soil were estimated using the dilution plate technique (Johnson *et al.* 1959). Wet soil was used, but the moisture content was assumed to be the same as that in the sample used to determine moisture content. Three stock solutions of 1:100 were prepared for each soil sample by weighing 1 g of wet soil into 100 ml of sterile distilled water. From these stock solutions, serial dilutions of 1:1000, 1:10000, and 1:100000 were prepared. Fungal colony forming units (CFUs) were made by pipetting and spreading 0.2 ml of an appropriate dilution (yielding 30-300 CFUs per plate) onto standard potato-dextrose agar (PDA) media with 50 mg of chloramphenicol per litre agar added. Three plates were used for each dilution, and were incubated at 38^{0}C for 4 days during which they were examined daily. The average CFU count was multiplied by the dilution factor to estimate the average number of CFUs per gram of soil for each soil sample. A set of blanks were also run for each batch of sample plates by inoculating three PDA plates with sterile distilled water instead of soil suspension. No fungi usually developed on these whereas liberal growth occurred on the soil inoculated plates. In some cases mycelium appeared on the very edge of the blank plates.

The counting of microbial colonies on dilution plates and calculation of numbers of organisms per gram of soil have been accepted as standard procedures in soil microbial studies, despite the consensus that we have no clear understanding of the meaning of plate counts. The majority of studies mentioned in this paper have dealt only with soil fungi obtainable from dilution plating techniques. Dilution plating techniques have been criticised on the grounds that spores give rise to most of the colonies, and this may result in species with low sporulation rates being obscured and/or missed. This is because vegetatively active mycelium may be underestimated using this method (Warcup 1955, 1960). However, the problems associated with the dilution plating technique are most applicable to community studies assessing relative abundance of species.

Air currents undoubtedly carry great numbers of fungal spores to any surface, including spores of species unable to survive on that substrate. However, such spores are probably attacked and eliminated very rapidly, either before germination or soon after (Christensen 1969). The fact that different assemblages of principal fungal species occur in different plant communities argues against the idea of continued growth and sporulation by unadapted species. In support of the dilution plating method, it should be pointed out that this method draws sample populations from a greater volume of soil than do most trapping methods. Species restricted to small fragments of organic or mineral matter are, therefore, not so easily missed (Christensen 1969). One of the defects in the dilution plate technique is that during the transfer of soil suspension by pipetting, heavy soil particles settle down quickly taking with them the suspended fungal propagules, greatly affecting the final count. A suspension agent was added to the stock dilutions to reduce this. This involved adding 0.05 ml of Tween 20 per 100 ml of soil suspension. Additionally, aliquots were taken from the centre of the suspension to standardise the procedure.

Air samples were collected from Mokoia using a SAS Spore Impacter with portable battery. Potato dextrose agar (PDA) plates were used to collect air samples. The spore impacter made it possible to collect standard 60 litre air samples from each of the soil sampling sites mentioned above. This involved placing a PDA plate into the spore impacter and holding the apparatus at shoulder hight with arm out stretched. Each sample took 20 seconds to impact 60 litres of air onto the PDA plate. After the sampling

procedure, plates were sealed with tape and transported back to the laboratory and incubated in the same manner as the soil samples. Colony counts were then divided by 60 to estimate number of *A. fumigatus* spores per litre air.

Air samples were collected from gully and edge locations on a monthly basis on Mokoia from October 2000 to April 2001. Sixty gully air samples were collected at the study sites used in the leaf litter removal experiments. Forty-nine edge air samples were collected at the forest boundary zone on the south/south-west side of the island (i.e., Hotpool, Hut, Flat areas, Figure 1). No air samples were collected during the January 2001 field trip due to equipment problems in the field.

Hobo dataloggers were used continuously throughout the experiment to record ground temperature and relative humidity at each study site. Because my data were highly skewed towards low counts, log transformation was required before analysis of variance could be performed. The residuals subsequent conformed closely to a normal distribution. Three-way ANOVA was used to test whether *A. fumigatus* densities varied according to the site, month, and treatment cage *vs* control cage. Additionally, data were tested according to whether seasonal variations in *A. fumigatus* were similar between treatment and control cages.

Results

Ground temperature and relative humidity at the experimental sites
Recorded ground temperatures ranged from 3.0-26.0°C, with a combined annual mean of 13°C. Ground temperatures varied significantly between sites (P< 0.01) and months (P< 0.01). Ground temperatures were highest in February (mean 17°C) and lowest in August (mean 9°C), and the seasonal changes were very similar among sites (Figure 3.2a). The mean differences between sites was that sites in Queen St gully were cooler (11.9-12.1°C) than other sites (13.1-13.4°C).

Relative humidity varied significantly between seasons (P = 0.03) and between some sites (P = 0.05). Mean RH was consistently high from April to September, averaging 96% over this period, but also fairly high in December and January. It was lowest from

Location	Cage	1 gram Soil samples	Total Plate counts	Soil moisture %H$_2$O g^{-1}	St. Error %H$_2$O g^{-1}	Mean CFUg^{-1}	St. Error CFUg^{-1}	Annual mean Ground Temp (°C)	Mean Max. Temp (°C)	Mean Min. Temp (°C)	Annual mean Rel. Humidity (%)	Mean max. Rel. Humidity (%)	Mean min. Rel. Humidity (%)
Hotpool	Treatment	72	216	30	3.0	487556	197329						
	Control	72	216	29	3.2	307299	60271	13.4	26.0	5.2	90.1	96.4	79.8
Totara Grove	Treatment	72	216	28	2.8	325037	84577						
	Control	72	216	28	2.3	197107	38058	13.1	25.6	6.6	88.6	95.3	79.8
Lower K-Gully	Treatment	72	216	35	1.4	431528	132891						
	Control	72	216	33	1.7	309144	111355	13.2	25.5	1.2	91.0	97.7	79.8
Upper K-Gully	Treatment	72	216	30	1.4	254398	58320						
	Control	72	216	30	1.3	295674	65014						
Lower D-Gully	Treatment	72	216	39	2.9	337060	128573						
	Control	72	216	42	2.5	375590	144446	13.6	26.4	5.8	90.2	96.6	78.1
Upper D-Gully	Treatment	72	216	34	3.0	402272	158494						
	Control	72	216	36	2.3	377887	190975						
Upper Queen St Gully1	Treatment	72	216	35	2.5	372187	123978						
	Control	72	216	36	1.3	293425	78440	11.9	26.0	4.1	89.7	99.9	82.0
Upper Queen St Gully2	Treatment	72	216	30	1.8	390574	84401						
	Control	72	216	32	1.9	284962	69316						
Mid Queen St Gully	Treatment	72	216	37	3.2	246681	32793						
	Control	72	216	45	2.5	155355	20633						
Lower Queen St Gully	Treatment	72	216	39	3.0	170366	23823	12.1	26.0	2.9	92.4	97.4	78.5
	Control	72	216	39	3.5	129465	21459						
Isabel's Camp	Treatment	72	216	22	1.3	769000	228000						
	Transects	72	216	27	3.0	445000	184000	13.7	26.4	3.2	89.0	96.2	78.0
Total		1584	4752										

Table 3.1. Summary of results for each treatment and control site set up for the leaf litter removal experiments on Mokoia Island from May 2000 to April 2001.
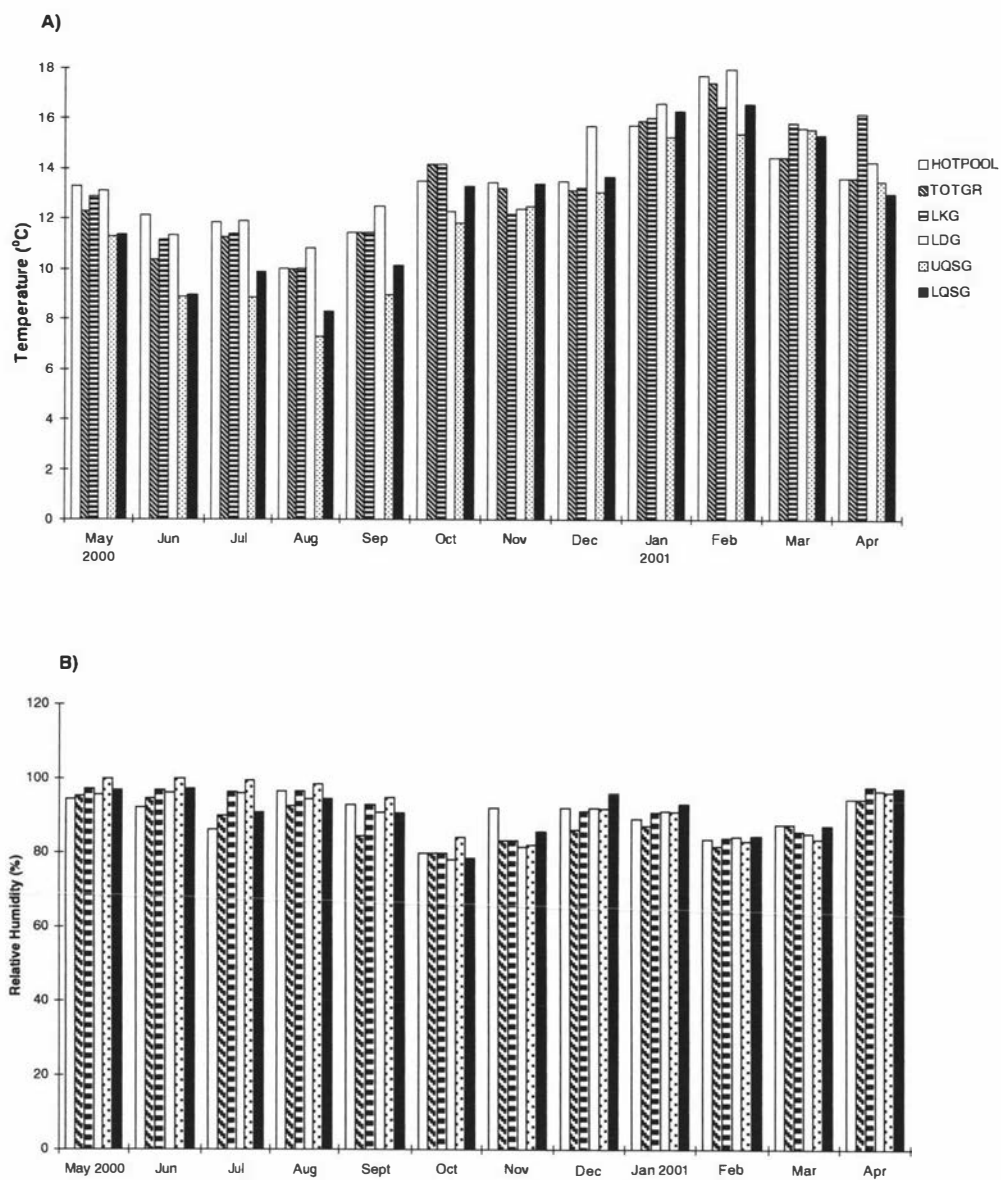
Figure 3.2. Monthly changes in A) mean ground temperature, and B) mean relative humidity at the sites used in the leaf litter removal experiments on Mokoia Island.

October/November (80%) and February/March (83%). The Totara Grove and Hotpool study sites had the lowest mean RH, and these were the sites closest to the forest edge.

Effect of leaf litter removal and season on Aspergillus fumigatus *densities*

Soil samples collected one month prior to the start of the leaf litter removal experiments had a mean *Aspergillus fumigatus* density of 315,000 ± 117,000 CFUg^{-1}. Densities at the conclusion of the leaf litter removal experiments were 342,000 ± 103,000 CFUg^{-1} and 273,000 ± 78,000 CFUg^{-1} respectively for treatment and control cages.

There were significant differences in the mean densities of *A. fumigatus* between sites (P <0.01), between months (P <0.01), and between treatment and control cages (P<0.01). For treatment and control cages combined, *A. fumigatus* density estimates ranged from zero to millions of colony forming units per gram of dry surface soil (CFUg^{-1}), with a combined mean and standard error of 308,000 ± 91,000 (Figure 3.3).

At the start of the leaf litter removal experiment, *A. fumigatus* densities were not significantly different between treatment and control sites, and the mean density was slightly (12%) higher at the control sites. The situation had reversed one month later, with treatment cages on average being 24% higher than control cages. During this period, *A. fumigatus* densities in the treatment cages increased by 18% from 292,000 ± 45,000 to 309,000 ± 35,000 CFUg^{-1}, while densities in the control cages dropped (49%) from 374,000 ± 73,000 to 192,000 ± 48,000 CFUg^{-1}. This dip in control counts can be attributed to seasonal change, as control counts continued to drop at most sites. The lack of change at treatment sites can therefore be attributed to the effect of exposing the surface soil layer. Treatment sites continued to have significantly higher (P< 0.01) densities than control sites for the subsequent 10 months except for summer (Dec-Feb) when levels were low at all sites. Treatment and control sites both showed strong seasonal changes in *A. fumigatus* densities, and these were closely correlated (Figure 3.3). *Aspergillus fumigatus* densities showed a biannual cycle, with peaks in spring and autumn. The levels, initially decreased until July (mid winter), then increased until they reached their peak in October (mid spring). They then decreased until they reached their lowest in January (mid summer), then rose until April (mid autumn).
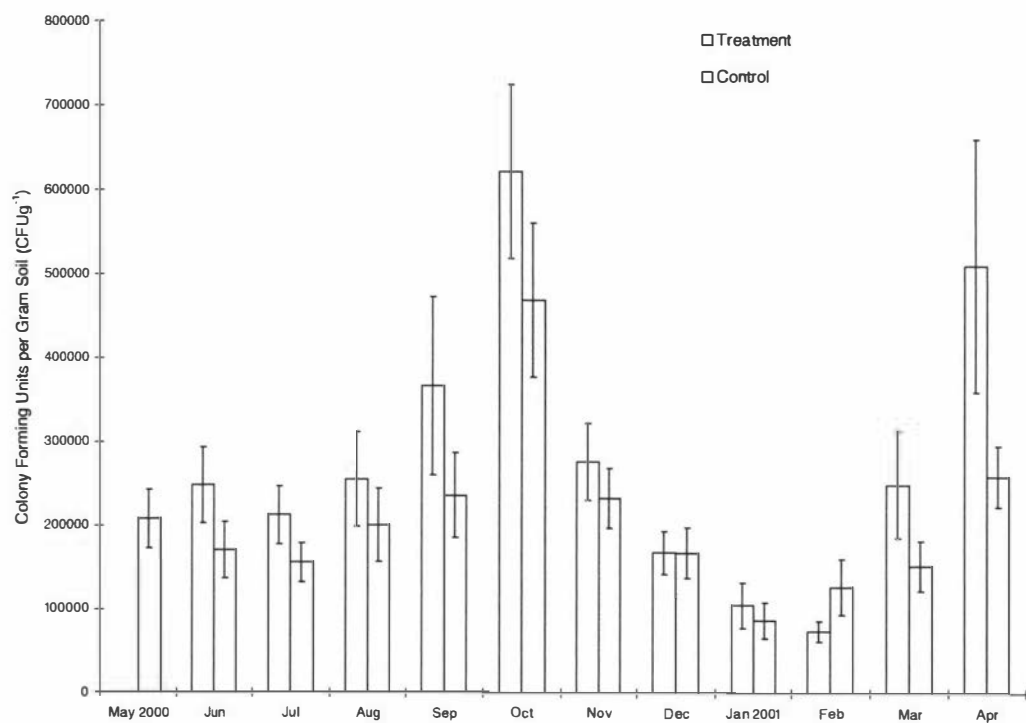
Figure 3.3. Monthly variations in *Aspergillus fumigatus* densities recorded at treatment and control sites on Mokoia Island. Bars indicate standard error.
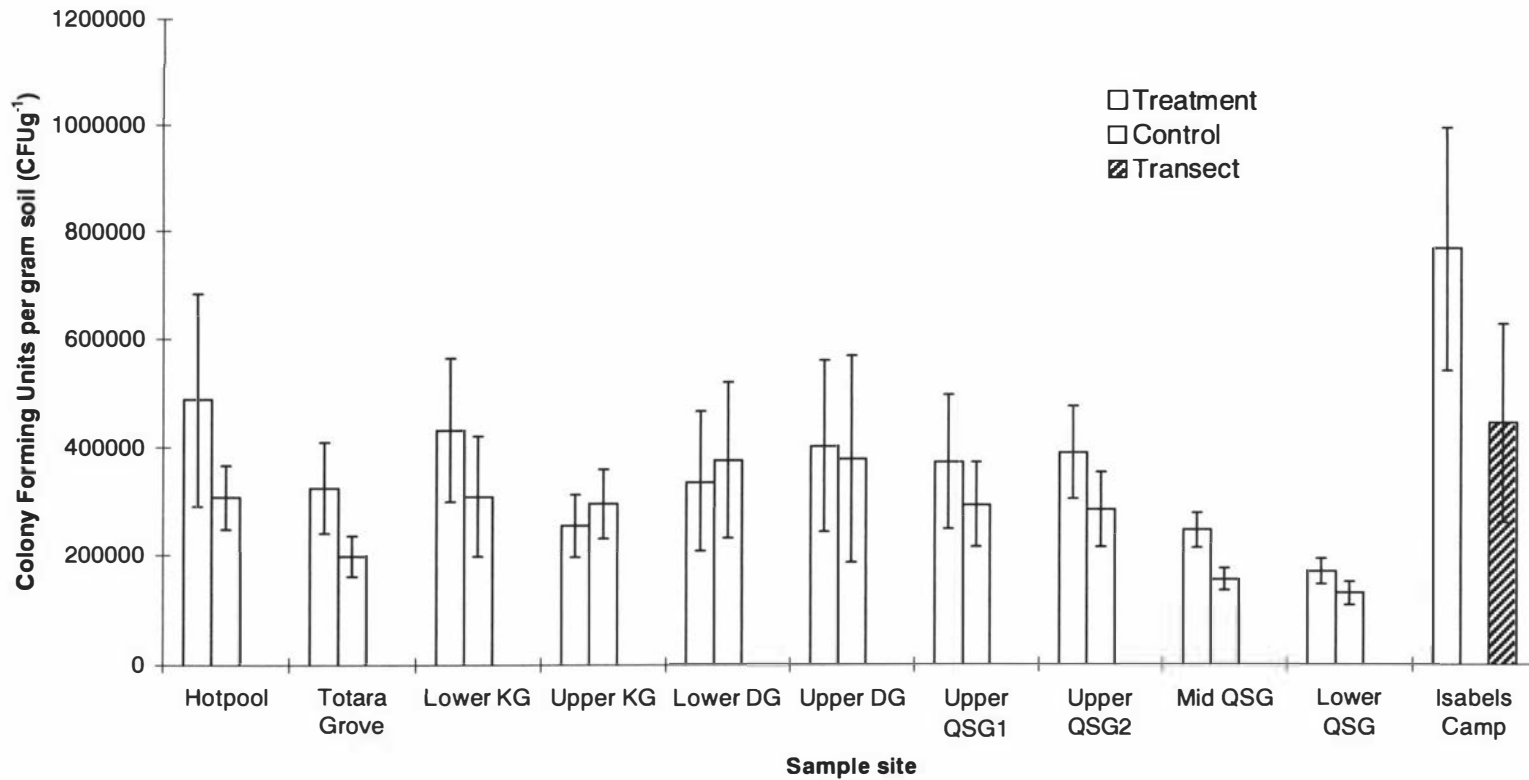
Figure 3.4. Total mean *Aspergillus fumigatus* densities for paired treatment and control cages at each sample site on Mokoia Island. Bars indicate standard error. Transect lines were taken in place of control cages at the Isabel`s Camp site.

Aspergillus fumigatus *densities between sites*

Among the treatment sites set up for this experiment, the highest *A. fumigatus* densities were recorded at Isabel's Camp site (769,000 ± 228,000 CFUg^{-1}) and the Hotpool study site (488,000 ± 197,000 CFUg^{-1}). The highest estimate for the control cages came from the Upper D-Gully study site (378,000 ± 158,000 CFUg^{-1}). The lowest estimates for the treatment and control cages both came from the Lower Queen St Gully site (Treatment = 170,000 ± 24,000 CFUg^{-1}; Control = 129,000 ± 21,000 CFUg^{-1}).

The mean density of *A. fumigatus* was higher for the treatment cages at eight of the eleven sites (Figure 4). The sites with the greatest proportional differences between paired treatment and control cages after one year were, Totara Grove (P = 0.09; 24%), Hotpool (P = 0.18; 22%), and Mid Queen St Gully (P = 0.01; 22%), Lower K-Gully (P = 0.01; 16%), Upper Queen St Gully2 (P = 0.05; 16%), Lower Queen St Gully (P = 0.05; 16%), and Upper Queen St Gully1 (P = 0.10; 12%). Three of the ten experimental sites showed little difference between treatment and control cages after one year, i.e., Upper D-Gully (4%), Lower D-Gully (7%), and Upper K-Gully (8%). Two years following disturbance in treatment cages at Isabel's Camp, *A. fumigatus* densities were still significantly higher compared to estimates from soil samples collected along transect lines in the same bush patch (P = 0.03).

With the exception of Isabel's Camp, Hotpool and Lower K-Gully, high elevation sites (see Figure 1) had higher *A. fumigatus* colony counts compared to sites situated near the shoreline (P <0.01). Sites situated on the western side of the island away from the shoreline tended to have higher mean *A. fumigatus* densities, and lower significance between treatment and control cages. Sites located near the bush edge and hut area (Hotpool and Isabel`s Camp) had higher levels compared to sites situated in gullies.

Effect of season on Aspergillus fumigatus *densities in the air*

In total, 109 air samples were collected on Mokoia over a seven-month period from late spring to mid autumn (Table 3.1, Figure 3.5). The density of airborne *A. fumigatus* spores ranged from 0 to 0.73 CFU per litre of air (CFUL^{-1}), with a total mean density of 0.36 ± 0.12 CFUL^{-1}. Mean *A. fumigatus* airborne spore densities varied significantly between months (P = 0.01). Airborne spore densities were highest in October (0.36 CFUL^{-1}) and dropped over summer reaching 0.11 CFUL^{-1} in March. The decrease in
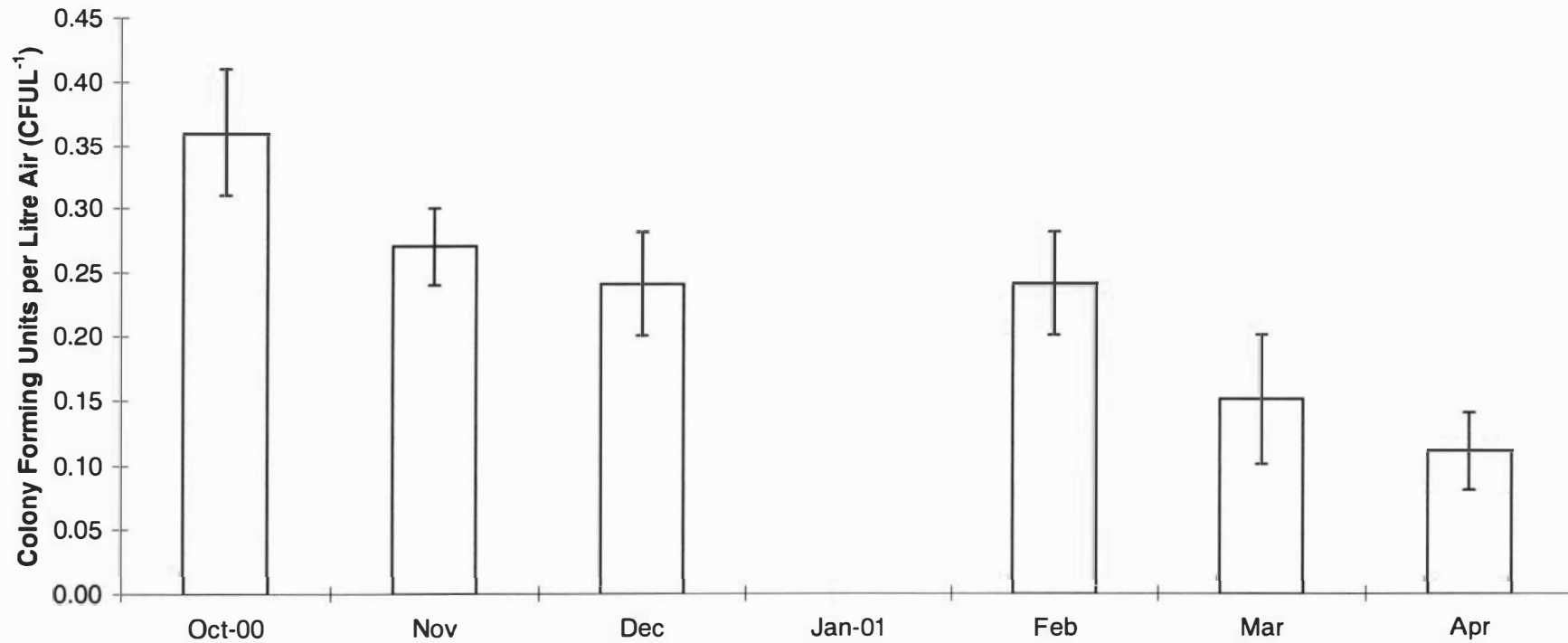
Figure 3.5. Monthly variations in *Aspergillus fumigatus* airborne spore densities on Mokoia Island. Bars indicate standard error.

airborne spore densities over autumn constrasts with the *A. fumigatus* densities in the surface soils, which increased over the same time period.

Discussion

This study has shown that removal of leaf litter increases the establishment of *A. fumigatus* in the surface soil layer. Additionally, results indicate high persistence of *A. fumigatus* two years following the disturbance event. There are probably many reasons why leaf litter removal may cause an elevation in *A. fumigatus* densities in the surface soil. The first causal factor could simply be increased access to the surface soil layer by airborne *A. fumigatus* spores. The leaf litter layer physically prevents airborne spores from reaching the surface soil layer (i.e., physical exclusion). Therefore, by exposing the surface soil layer to the air I may have increased the potential for airborne spores to colonise the surface soil layer. Secondly, exposing the surface soil layer to the desiccating effects of dry air may benefit *A. fumigatus* by creating conditions in the surface soil that exclude less drought/heat tolerant microbes (i.e., habitat exclusion). Thirdly, *A. fumigatus* may have a competitive advantage over other fungi in disturbed habitats. Reduction in the density of other microbes may provide *A. fumigatus* with greater opportunities for growth and persistence in these habitats.

Hyphal material is usually short lived in the soil (Starkey 1938; Atlas and Bartha 1997) because they are quickly attacked by other microbes. In various species-specific mycological studies a correlation has been found between the abundance of spores and the abundance of mycelium (e.g., Witkamp and van der Dritt 1961; Burges and Nicholas 1962). I therefore, assume that the frequency of spores, measured over time, is indicative of fungal activity in the soil on Mokoia.

Microbial activity and densities are primarily controlled by moisture, temperature, and the nature of the organic substrate/s. These factors are interrelated as both moisture and temperature affect the supply of organic substrates available to fungi. My results indicate that *A. fumigatus* densities in the soil show well-marked periodicity throughout the year, with maximum densities in autumn and spring. According to Hawker (1950), the moisture requirements vary greatly among different types of soil fungi. In this study,

colony counts dropped significantly over the summer months. This suggests that drying out of the leaf litter and surface soil over summer limits the density of *A. fumigatus* in that habitat. It is important to remember that the production of spores by soil fungi, although primarily a reproductive strategy, also provide a means for survival against adverse soil conditions.

Hepple (1960) suggests that short periods of waterlogging, followed by drainage, may have considerable ecological significance in determining the composition of the soil mycoflora. My data suggest that short periods of desiccation, followed by periods of waterlogging and drainage, may also have considerable ecological significance in determining the density of *A. fumigatus* in the surface soil. It is interesting to speculate on the functional significance of the surface soil habitat to weedy fungi such as *Aspergillus*. Over summer, rapid drying out of the leaf litter and surface soil layer renders this habitat almost devoid of all 'active' life. However, as soon as soil moisture levels increase following summer this habitat is available again to recolonisation by saprophytes. Cellulose degrading fungi such as the *Aspergillus* group, which invest heavily in dispersal, would seem ideally adapted to take advantage of this seasonal disturbance regime. It may be the stochastic nature of the disturbance process, rather than the drought conditions themselves, that provides *A. fumigatus* with a selective advantage in these environments. This type of annual habitat variation may allow *Aspergillus* levels to remain high by providing conditions that promote continued recolonisation of surface substrates. If true, this may suggest that naturally disturbed habitats, such as coastal and forest edge locations, may typically have high levels of *A. fumigatus*. This is because these habitats are notoriously transient in nature and primarily driven by externally generated disturbance events. In this way, *Aspergillus* infestations are the results of other disturbances as well as being disturbances in their own right.

The complete destruction of our lowland forest ecosystem in New Zealands has left us with a highly modified fragmented landscape, dominated by forest edge habitat. This causes forest patches and native species to become vulnerable disturbance events. This in turn changes the disease dynamics in these areas by increasing the prevalence of opportunist disease causing agents such as *A. fumigatus*, and opportunist insect host/vector species. Insects such as juvenile cicada nymphs spend the majority of their

life cycle underground, and are found in higher densities in young regenerating habitats such as secondary growth forests and forest edge habitats (White 1980). This is because adult female cicadas prefer to lay their eggs in young regenerating vegetation (2-3 year old twigs), and these tend to be more abundant in young growth forest edge habitats (White 1980, Williams and Simon 1995, see Chapter 1). Every year, multitudes of fifth instar cicada nymphs emerge from their subterranean burrows and climb the nearest vertical surface to shed their last exoskeleton, develop wings, and start their adult phase. Each cicada nymph carries on it small amounts of soil adhering to its exoskeleton, which it transports to the surface. *Aspergillus fumigatus* is well known for it ability to utilise chitin and for its adaptations to wind dispersal (St Leger and Screen 2000). On Mokoia during November 2000, 100 discarded cicada exoskeletons were collected shortly after emergence and washed collectively in 20 mls of distilled sterile water with Tween 20 to remove all soil particles. The soil and water solution was then slowly evaporated over several days and the remaining soil material dry weighed. I found that on average each exoskeleton had about 0.012 g of dry soil material attached to its exoskeleton post emergence. It is likely that this estimate would be influenced greatly by soil type and weather conditions prevailing at the time (Witkamp and van der Drift 1961). On Mokoia, fifth instar cicada nymphal emergent rates were estimate at 93 cicadas per square metre during the 1998/1999 emergence period (i.e., the previous year). This equates to a mean soil turnover rate of about 0.9 grams of soil per square meter, or about 90 grams of soil per hectare. Factoring in the estimated levels of *A. fumigatus* per gram of soil on Mokoia at the time of emergence (i.e., 445000 ± 35000 CFUg^{-1}) this equates to an annual soil turnover of about 40 X 10^6 CFUs of *A. fumigatus* per hectare caused by cicada emergence alone.

Estimated soil turnover rates caused by cicadas on Mokoia are given as a basic example of another predictable natural disturbance event that can potentially contribute to high densities of *A. fumigatus* in forest edge habitats. When you consider the multitude of organisms and unnatural disturbance events that can potentially contribute to soil turn over rates, it is likely that the actual soil turnover rates on Mokoia are many times higher than those caused by cicada emergence alone. However, it is not just soil turn over on its own that benefits *A. fumigatus* dispersal rates, but also the position of the exoskeleton discarded above the forest floor which probably increases the wind dispersal potential of *A. fumigatus* as well.

The investigations recorded in this paper on the ecology *A. fumigatus* on Mokoia Island have been of a general nature, and, of necessity, many interesting questions remain untouched. The limited data show that specialised habitats and ecological processes are important and must be studied to obtain a full understanding of the occurrence and activity of *A. fumigatus* in forest soils. The definition of "disturbance", and what actually constitutes a disturbance event, are still debated. White and Pickett (1985) define a disturbance event as "any relatively discrete event in time that disrupts ecosystem, community, or the physical environment". Petraitis *et al.* (1989) included under disturbance any "process that alters the birth and death rates of individuals present in the patch". In this study the occurrence of *A. fumigatus* was estimated following a singular disturbance event, i.e., removal of all leaf litter at the beginning of the experiment. The impact of continued disturbance or disturbance during different seasons on the occurrence of *A. fumigatus* has yet to be evaluated.

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Chapter 4

Aspergillus fumigatus densities from forest soils, air, and flowers in relation to forest succession and edge effects

Introduction

Concerns about health effects from dry dusty conditions have tended to centre around *Aspergillus fumigatus* for several reasons. First, the spores of this fungus are heat and drought tolerant, and produced in great abundance (Atlas and Bartha 1998). Second, because of the small size of the asexual spores (conidia), they are easily dispersed into the air (Gravesen 1979; Millner et al. 1980; Liesivuori *et al.* 1994; Epstein 1996). Third, the conidia are small enough (i.e., 2 to 3 micrometres diameter) to reach the alveoli of the lungs when inhaled; and fourth, *A. fumigatus* is one of few fungal species able to grow and multiply within the respiratory system of immunocompromised people (Glimp and Bayer 1983; Vincken and Roels 1984; Wyngaarden and Smith 1988). *Aspergillus* infections can be acute and life-threatening in small animals, especially those suffering poor health at the time of exposure (e.g., Morey *et al.* 1989; Bauck 1994; Fogelmark *et al.* 1994), and can be complicated by other respiratory aliments such as asthma (Greenberger and Patterson 1988; Michel *et al.* 1992). However, infection is considered non-contagious in animals and plays no role in the prevalence of the fungus in the environment (Wobeser 1996).

The hihi (*Notiomystis cincta*) is a small (i.e., starling sized) cavity-nesting honeyeater endemic to New Zealand. Hihi seem to be particularly susceptible to *A. fumigatus* infections (e.g., Alley *et al.* 1999). Hihi became extinct on the mainland about 1885, and presently a single self-sustaining population of the species remains on Little Barrier Island (LBI). Attempts to establish hihi on other predator free islands have mostly been unsuccessful. During September 1994, 40 hihi were translocated from LBI to Mokoia

Island in Lake Rotorua. Seven years on, hihi on Mokoia have been reduced to six breeding females, about 14 males, and 5-9 fledglings (Griffiths 2001, Internal DOC Report to Hihi Recovery Group). From 1995 to 1997, 44% of hihi on Mokoia were reported to be showing clinical signs of illness suggestive of aspergillosis prior to death or disappearance. Additionally, post-mortem examination of six dead hihi on Mokoia from 1995-1997 identified the fungal disease aspergillosis as the most common cause of death among those birds (Alley *et al*. 1999). The only captive hihi population at the Mount Bruce Wildlife Centre also suffer high *A. fumigatus* infection rates (M.R Alley, *pers comm*.). By not establishing hihi populations outside Little Barrier Island the future of this species is uncertain. Because hihi may be particularly susceptible to aspergillosis there is great need for research on the occurrence of *Aspergillus* at sites that have resident hihi and sites that may be used for reintroduction attempts in the future.

In Chapter Two, I presented evidence suggesting that *A. fumigatus* densities are high on Mokoia Island compared to other studies (e.g., Moubasher and Abdel-Hafez 1978; Akpata and Ekundaya 1983, Smid *et al*. 1992). However, higher densities have been recorded from indoor environments (e.g. Wyngaarden and Smith 1988; Lavoie 1997). This may suggest that hihi on Mokoia are suffering high levels of exposure to *A. fumigatus* spores. From the leaf litter removal experiments I found evidence that habitat disturbance increases the establishment success of *A. fumigatus*. This suggests that modified landscapes and forest edge zones should be ideal *A. fumigatus* habitat, as they are subject to a greater variety of transient disturbance events compared to interior forest locations.

The edge of a forest patch constitutes a different habitat to the forest interior, typically having more weedy plant species, higher light availability, greater wind and frost damage, and higher day and lower night temperatures (Saunders *et al*. 1991; Kapos *et al* 1997; Turton 1997). The altered microclimate of the forest edge has been found to be unsuitable for many organisms, while promoting an increase in abundance of others beyond their native ranges (Wilson and Peter 1988). In some forests, edge zones may extend 30 m into the interior of the forest fragment (Ranney *et al*. 1981; Mitchell and Young 1994). If there exists a link between forest disturbance and the abundance of *Aspergillus*, then it is possible that habitat modification and fragmentation increases *A. fumigatus* contamination in the environment. Increased exposure to *A. fumigatus* spores

may predispose some bird populations and species to diseases that they historically had little contact with.

In this chapter, *A. fumigatus* densities are estimated from surface soils, flowers and air from forest edge zones, inner forest locations, and summit areas on Mokoia and Little Barrier Islands. Additional soil samples were collected from Tiritiri Matangi Island (Tiri), Mt Bruce Wildlife Centre, and Massey University Campus for comparison. This information is important because at present there are no comparative studies on the occurrence of *A. fumigatus* in New Zealand any forests. This chapter reports work designed to achieve the following objectives, 1) Compare densities of *A. fumigatus* on LBI, Tiritiri Matangi Island, and two mainland sites to Mokoia 2) Compare densities of *A. fumigatus* from forests edge habitats with inner forest gully locations 2) Independently test for *A. fumigatus* density forest edge effects using flowers as a sampling tool 3) Determine to what degree rainfall effects *A. fumigatus* densities in flowers.

Methods

Study sites

Soil sampling took place at five study sites in the North Island (Figure 4.1)

Mokoia Island is situated in Lake Rotorua in the North Island ($38^0 06`S$, $174^0 55`E$). With an area of 135 ha, it is the largest inland island in New Zealand (Figure 4.2). The shortest distance from the mainland is about 2.1 km. Mokoia is a small volcanic island (summit 154 m above lake level) and is composed mostly of regenerating native tree and shrub species (Perrott and Armstrong 2000). Mokoia has a long history of human occupation dating back several hundred years. A variety of domestic stock were farmed on Mokoia up until the 1950s (Andrews 1992). Over the last 40 years secondary forest has spread from the gullies and now covers most of the island. The vegetation is low and scrubby on the ridges, particularly near the summit, whereas there is a closed seral-mahoe canopy and open forest floor in gullies and near the lake shore. Subcanopy regeneration has occurred since 1989 when goats and rats were eradicated. The forest floor over most of Mokoia has a covering of leaf litter which is damp for most of the
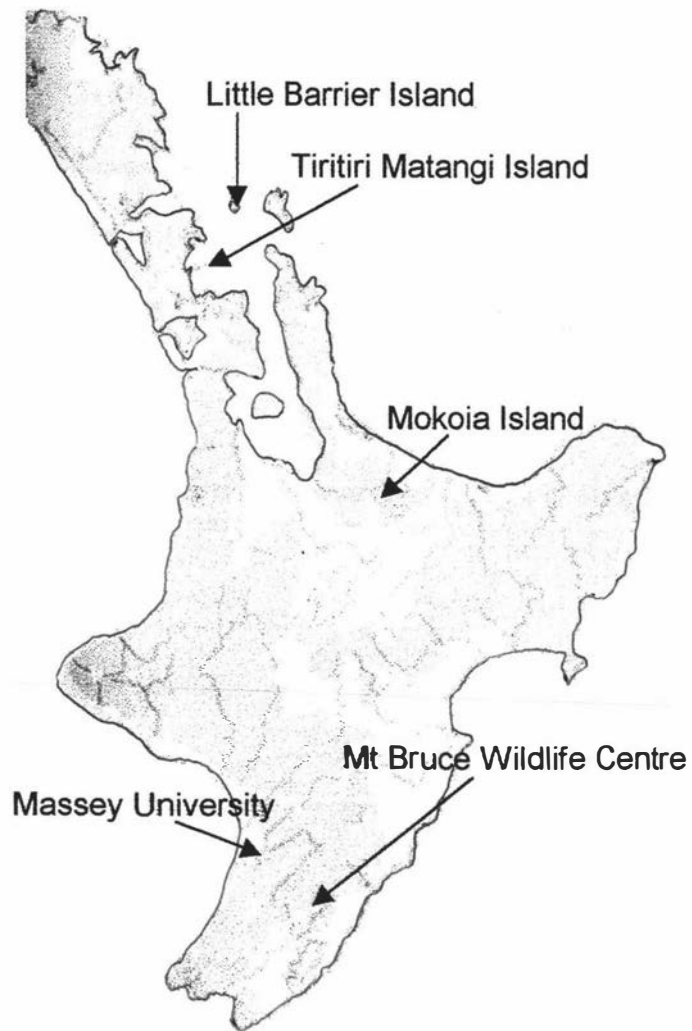
Figure 4.1 Map of North Island of New Zealand showing the locations of the study sites in this study.
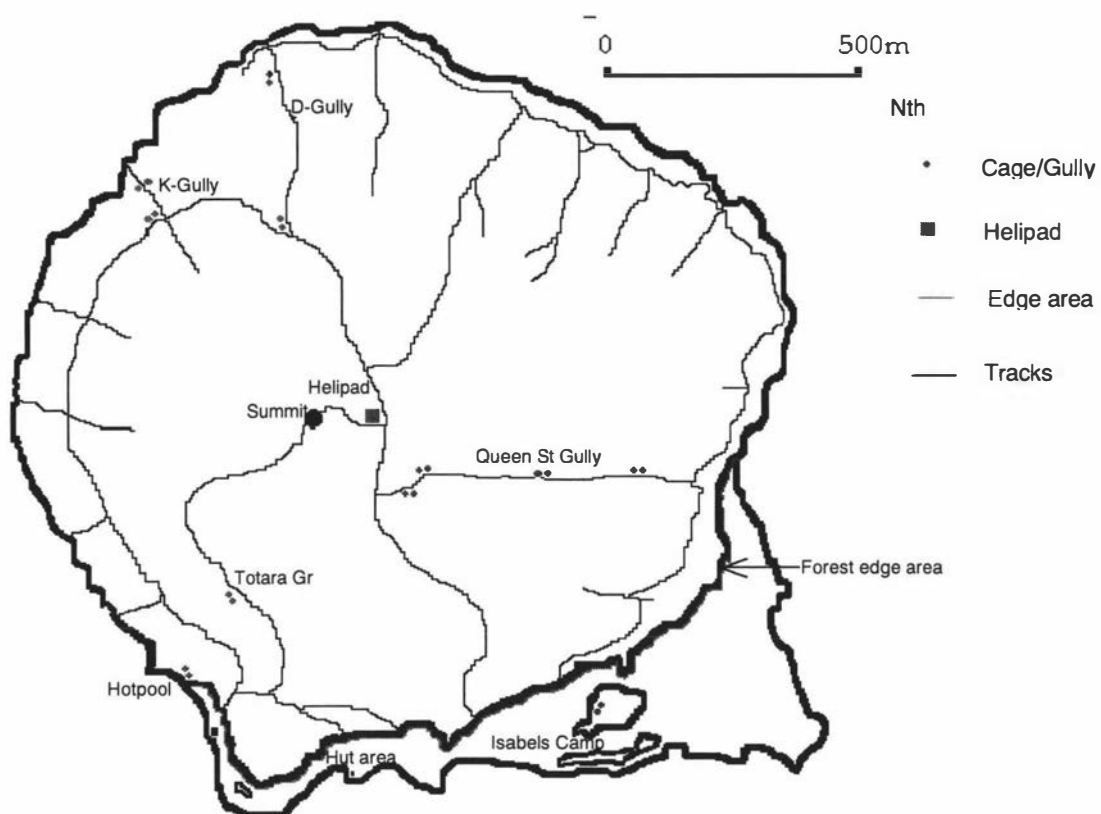
Figure 4.2. Map of Mokoia Island showing the locations of sample sites and tracks on the island.

year. During the summer months dry dusty conditions prevail and the leaf litter and soil surface layer dry out, especially outside gully areas. I sampled three gullies (K-Gully, D-Gully, and Queen St-Gully), the summit area (above 125 m), and three forest edge areas (i.e., Hotpool, Isabel's Camp, and the Helipad sites).

2) Little Barrier Island or Hauturu ($36^012`S$, $175^07`E$) lies approximately 24 km from Cape Rodney on the east coast of the North Island at the northern entrance to the Hauraki Gulf (Figure 4.3). The large and diverse LBI (3076 ha, 772 m above sea level) contains several mature forest types, and its high elevation forest areas have been untouched by logging activity. The lower third of the slopes are covered with *Leptospermum* (manuka) and *Kunzea* (kanuka) and were logged in the 1880s. LBI has never been browsed by deer, goats or possums, is mostly trackless, and is covered in thick forest with a well developed upper canopy (in places 40 m high). LBI is home to all native forest bird species that are widespread on the mainland, some in great abundance, plus the only natural population of hihi (or stitchbird *Notiomystis cincta*). It has kiore (Polynesian rat, *Rattus exulans*) and had feral cats until they were eradicated in 1980 (Hayward 1986). While birds like hihi use most forest types, they seem to favour the mature moist forests in the larger valleys and elevated areas 300 m above sea level (Angehr 1984). The study site was located on the southwestern quarter of the island and extended to 678 m above sea level. Areas covered in this study include, Tirikakawa Stream, Te Waikahare Stream, Hamilton Track, and Herekohu (near summit area).

3) Tiritiri Matangi (Tiri) is a 220 ha offshore island ($36^034`S$, $175^055`E$) in the Hauraki gulf, 3.5 km east of Whangaparaoa Peninsula and 28 km north of Auckland City. Tiritiri Matangi is highly modified, having had a long history of human occupation. The island was cleared last century, and converted to farmland with several remnant forest patches comprising about 25 ha remaining. Tiritiri Matangi was stocked with sheep and cattle until 1971, and kiore was found on the island until 1993. Most of Tiri is now mammal free (sheep are still grazed on one part of the island) and has successfully undergone extensive replanting of native trees and shrubs (Mitchell 1995, Cashmore 1995). Areas covered in this study involve eight forest fragments scattered across the island. Soil samples on this island were collected from bush patches with active hihi nests in the area. Soil samples were collected by finding an active nest box and walking 5 m away at
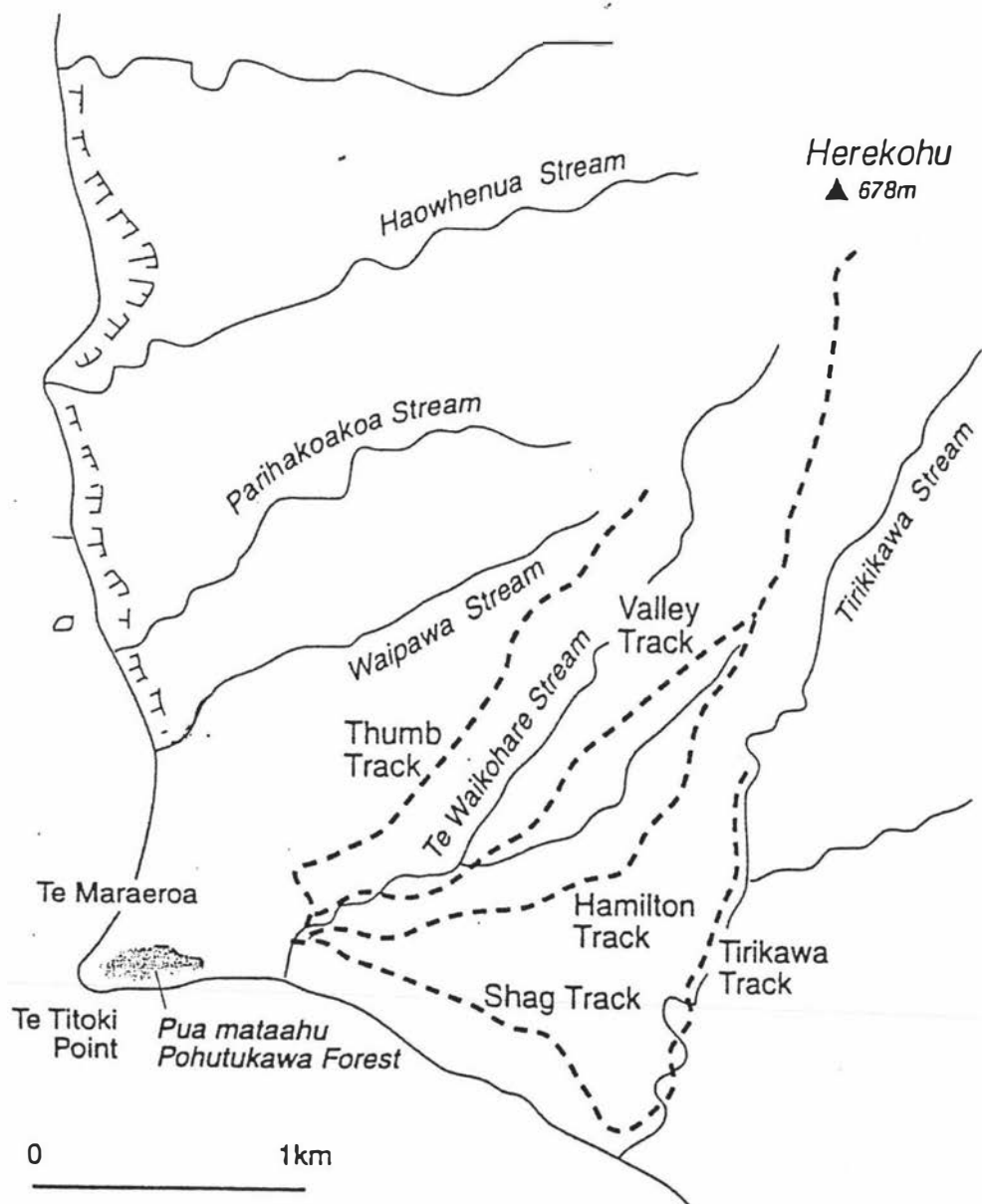
Figure 4.3 Map showing location of study area and tracks on Little Barrier Island (Hauturu).

a compass bearing likely to have the least human traffic, i.e., avoided anywhere someone was likely to walk.

4) Mt Bruce Wildlife Centre is situated in the lower North Island along State Highway 2 (28 km north of Masterton, $40^0 97`S$, $175^0 66`E$). The Mt Bruce Wildlife Centre is situated on the edge of a lowland forest fragment in the lower North Island near Masterton, and represents the southern most sample site in this study. Forests in this area were heavily logged for decades until the late 1940s, after which, the remaining forest fragment was protected and allowed to regenerate. Today, the forest is composed of several mature canopy species, such as totara, tawa, and some hand planted exotic tree species located around the wildlife centre. Areas sampled in this study involve five hihi aviaries and the forest immediately surrounding the aviaries.

5) Massey University Turitea Campus, is situated in Palmerston North in the lower North Island, in the Manawatu District ($40^0 35`S, 175^0 55`E$). Palmerston North is about 28 km inland from west coast and is surrounded by an agricultural landscape. Areas covered at this study site include the bush fragment opposite the Veterinary Science tower below Moginie Hall, and the bush fragment surrounding Turitea Stream at Bledisloe Park.

Sampling procedures

Substrates collected and screened for *Aspergillus fumigatus* included surface soil, flowers, and air. Samples were collected from five study sites and transported back to the Plant Pathology Laboratory in the Institute of Natural Resources at Massey University for processing, usually within 1-4 days of collection. For the purpose of this chapter, Mokoia and LBI data are grouped into three categories, (i) forest edge zone, the zone of habitat within 30 m of forest boundary, (ii) forest interior, the zone between summit and forest edge, and (iii) summit area, the zone within 30 m of highest point of elevation. Tiritiri Matangi does not have a summit, and all samples were > 30 m from the forest edge, so they are all considered to be forest interior samples. All soil samples collected near hihi aviaries at the Mt Bruce Wildlife Centre and at Massey University were collected < 30 m from the forest edge, so they are all considered to be forest edge samples. However, the soil sampling and isolation methods are the same between the study sites, and identical to those reported in Chapter 2.

A) Soil and leaf litter

Surface soil and leaf litter (about 50 g per sample) was removed to a depth of 2 cm from each sampling site. On Mokoia interior soil samples are derived from control cage estimates used in the leaf litter removal experiments (Chapter 2). Edge zone sample areas included the Hotpool area, the Hut area including the forest boundary along the south-eastern flat (Kaiweka) and Isabel`s Camp site, and the Helipad sampling area near the summit. The Helipad sampling site on Mokoia is a 35 m^2 circular clearing cut out of the bush in late July 2000. All vegetation and surface debris were removed in the construction of this site and soil left exposed over spring and summer. Summit soil samples were collected at 10 m intervals along 30 m transects lines at specific compass bearings descending from the highest point of elevation. This sampling area included the Helipad site prior to the disturbance event. On a monthly basis after the Helipad site was made, transect lines were taken from the centre of the clearing into the surrounding forest.

On Little Barrier Island, the selection of sampling sites were also spilt into, forest edge, gully and summit areas. However, the initial selection of these sites were determined by the sighting or hearing of hihi in the area. The rationale was to associate the soil sampling with the presence of hihi to be consistent with other sites. When a hihi was heard or seen we moved to the bird`s approximate location. From this point a surface soil sample was collected using the same methods as above. These sites were all gully areas away from the edge, so 5 X 100 m transect lines were made from the edge surrounding the hut and Rangers residence into the forest. During this procedure we stopped every ten meters and collected 1 X 50 g surface soil and leaf litter sample. Because the upper summit area of LBI (Hauturu, 722 m) is sacred/tapu to Ngatiwai, soil samples were collected along transect lines below this point at Herekohu (678 m). Samples were collected during late November 2000, early March 2001, and early May 2001.

Samples on Tiriiri Matangi were collected from 25 interior forest localities spread across the island. Soil and leaf litter samples were collected in one day during Nov 2000.

At the Mt Bruce Wildlife centre samples were collected from inside and outside five hihi enclosures during a one day visit in late January 2001. Samples inside enclosures were taken from the four corners and from the centre. Samples outside enclosures were collected at 5 m intervals along transect lines, starting outside each hihi enclosure and leading 20 m into the surrounding forest edge zone.

Samples from Massey University were collected from August 2000 to February 2001. I sampled a 20 m transect at each area each month, collecting five soil samples from each transect.

In the laboratory soil samples for determinations of moisture content were crushed by careful hand manipulation. Estimates of the numbers of *A. fumigatus* propagules per gram of dry soil and leaf litter were arrived at through use of the dilution plate technique (Johnson *et al.* 1959). Three stock solutions of 1:100 were prepared for each soil sample collected by weighing 1 g of wet soil into 100 ml of sterile distilled water. Therefore, each soil sample collected in the field was subsampled three times in the laboratory, ultimately providing at least 6 colony plate counts over three dilutions. Leaf litter material was blended for 5 minutes to obtain three 1:100 stock solutions. From these stock solutions three serial dilutions of 1:1000 and 1:10000 and 1:100000 were prepared in the same manner as the soil samples.

Fungal colony forming units (CFUs) were estimated by pipetting and spreading 0.2 ml of an appropriate dilution (yielding 30-300 CFUs per plate) onto standard potato-dextrose agar (PDA) media with 30 mg of chloramphenicol per litre agar added. Three plates were used for each dilution, and were incubated at 38^0C for 4 days during which they were examined daily. The average CFU count was multiplied by the dilution factor to estimate the number of CFUs per gram of soil for each plate count sample. A set of blanks were also run for each batch of sample plates by inoculating three PDA plates with sterile distilled water instead of soil suspension. No fungi developed on most blanks, whereas liberal growth occurred on most soil inoculated plates. In the few cases in which fungal colonies developed on blanks, it consisted of mycelium at the very edge of the plate. One of the defects of the dilution plate technique is that during the transference of soil suspension by pipetting, heavy soil particles settle down quickly, taking with them the suspended fungal propagules, and this affects greatly the final

count. To reduce this, a suspension agent was added to the stock dilutions. This involved adding 0.05 ml of Tween 20 per 100ml of soil suspension. Additionally, to standardise the procedure aliquots were taken from the centre of the suspension.

Soil and leaf litter moisture content was estimated for each soil sample by removing 1g of wet soil and drying this in an oven at 37^0C to constant weight (2 to 3 days). Dry soil weight was then calculated from the wet/dry weight difference. This was then factored into the final plate counts to estimate the number of fungal colonies per gram dry soil and leaf litter.

B) Flowers

Flower samples were used to test for edge effects and the influence of rainfall on *A. fumigatus* levels in flowers. Flowers were collected from Mokoia Island, LBI, and Massey University in association with the soil sampling. My target species were *Fuchsia excorticata* (Kotukutuku), *Pittosporum tenuifolium* (Kohuhu), *Pseudopanax arboreus* (Five-finger), and *Geniostoma rupestre* (Hangehange). These species were targeted because they are widespread on Mokoia, and known to grow along the forest edge and within the forest interior. No flowers from the target list were collected from LBI, Tiri, or Mt Bruce Wildlife Centre. Kohuhu and hangehange flowers were collected on campus at Massey University from August to December 2000.

Edge flowers on Mokoia were collected by walking within 10 m of the bush edge from the Hotpool study site to the entrance of Queen St Gully (about 500 m). Target trees were marked and samples collected monthly during their flowering periods. Samples from trees in the interior were made while moving about the island. When a plant was sampled, 5 to 10 flowers were selected randomly and placed separately in sterile containers. At least five plants per category were sampled each time. For those species such as five-finger that condense their flowers into inflorescences the individual flowers were taken randomly from at least five inflorescences per plant.

Kohuhu flowers collected on the Massey University Campus were used to test for the effects of rainfall on the levels of *A. fumigatus* found on or in flowers. Old flowers were removed by shaking the branches prior to sampling. On September 2000, it had not rained for over 2 weeks at Massey University. During this time 41 flowers were

randomly selected and removed from two kohuhu trees. These were screened for *A. fumigatus* on the same day of collection. On the August 28th 2000, it started to rain heavily on campus and lasted until October 1st 2000. On the day the rain stopped, another 41 flowers were collected and again screened for *A. fumigatus*.

Flower samples collected were transported back to the laboratory and screened for *Aspergillus fumigatus*. This involved removing each flower from its container and placing it in a test tube with 10 ml of sterile distilled water (with 0.01% Tween 20) at room temperature. Sterile cottonwool was used to stop the test tubes. Test tubes were then agitated for 5 mins in an electric vortex. From these stock solutions five 2 ml aliquots were removed and spread onto five PDA plates using the same methods outlined above. Colony plate counts were then multiplied by 5 to estimate number of CFUs per flower.

C) Air

Air samples were collected from forest interior and edge locations on a monthly basis on Mokoia from October 2000 to April 2001. Air samples were also collected from LBI, and the Massey University Campus. A SAS Spore Impacter with portable battery and PDA plates were used to collect air samples. The spore impacter made it possible to collect standard 60 litre air samples from each of the soil sampling sites mentioned above. This involved placing a PDA plate into the spore impacter and holding the apparatus at shoulder hight with arm out stretched. Each sample took 20 seconds to impact 60 litres of air onto the PDA plate. Sixty forest interior air samples were collected at the study sites used in the leaf litter removal experiments (Chapter 2). Forty nine edge air samples were collected at the Helipad study site, and the forest boundary zone on the south/south-west side of the island (i.e., Hotpool, Hut, Flat areas). No air samples were collected during the January 2001 field trip due to equipment problems in the field. After the sampling procedure, plates were sealed with tape and transported back to the laboratory and incubated in the same manner as the soil samples. Colony counts were then divided by 60 to estimate number of *A. fumigatus* spores per litre air (i.e., $CFUL^{-1}$).

D) Statistical analysis

The pairwise significance of the differences between sample sites were analysed using a Student's t-test of significance, and means differences were treated as significant at a probability of 5% or below. Because of limitations in the sampling design, I recognise that care is needed in comparing statistical results between study sites

Results

Soil and leaf litter

On Mokoia, total mean *A. fumigatus* densities from control cages (i.e., interior), edge and summit transect lines ranged from zero to 6,000,000 CFUs per gram soil with a combined average of $400,000 \pm 94,000$ CFUg^{-1} (Figure 4.4). Total mean densities of *A. fumigatus* from control cages reported in Chapter 2 (i.e., $273,000 \pm 30,000$ CFUg^{-1}) are about 20% below the mean estimates reported in this chapter. This is because the data set for this chapter is supplemented with 120 transect soil samples collected along bush edges and the summit area on Mokoia. Additionally, the data set for this chapter does not include any treatment cage estimates, and the Totara grove site is excluded because it is neither a gully, edge or summit location.

Little Barrier Island estimates ranged from 0 to 550,000 CFUg^{-1}, with a total mean average of $41,000 \pm 8,000$ CFUg^{-1} (Figure 4.4). A high proportion of plate counts returned zero colony counts (52%), and only 17% of the plate counts were over 100,000 CFUg^{-1}. In contrast, on Mokoia only 4% of plate counts returned a zero estimate, and over 75% of plate counts were over 100,000 CFUg^{-1}. On Tiritiri Matangi estimates ranged from zero to 1,200,000 CFUg^{-1}, with a total mean of density of $158,000 \pm 37,000$ CFUg^{-1}. From these data, 25% of colony counts returned zero estimations, while 55% of the plate counts gave estimations above 100,000 CFUg^{-1}.

Comparing the three islands, it is clear that LBI has significantly lower levels of *A. fumigatus* than both Mokoia and Tiritiri Matangi ($P < 0.01$). Additionally, because of the high proportion of zero counts it is likely that *A. fumigatus* on LBI has a patchy distribution. This is in contrast to Mokoia where few counts fell below 100,000 CFUg^{-1} indicating a more uniformed distribution over the island. The maximum estimate for
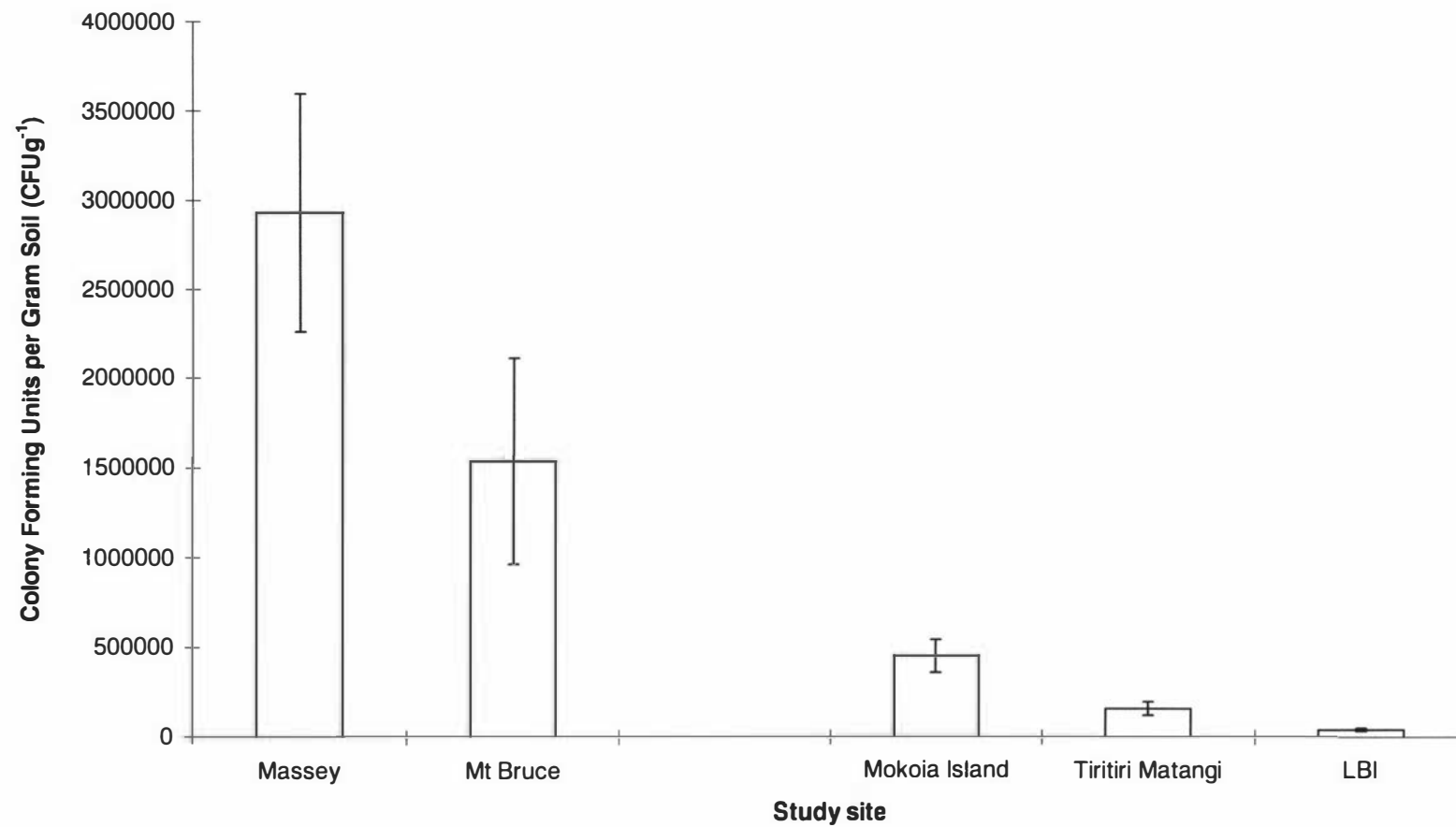
Figure 4.4. *Aspergillus fumigatus* densities in the soil on Mokoia, Tiritiri Matangi, and Little Barrier Islands, and two mainland study sites, Massey University Campus and the Mt Bruce Wildlife Centre. Bars indicate standard error.

LBI was above the means for both Mokoia and Tiri, which indicates that for some localities on LBI, *A. fumigatus* levels are comparable to Mokoia and Tiri. On Tiri the range is about twice that of LBI and the mean density estimation about 8 times higher (P < 0.01). Compared to Mokoia, the range on Tiri is smaller, but samples from both islands range into the millions of moulds per gram soil. However, the mean density on Mokoia is about 3 times higher than that recorded on Tiri (P < 0.01). This indicates that while Tiri has mean densities significantly below those recorded on Mokoia, there are a moderate amount of sites on Tiri with levels above the mean densities recorded on Mokoia.

The two mainland sites (Mt Bruce and the Massey University Campus sites) are significant for having mean *A. fumigatus* densities in the millions of moulds per gram of soil (Figure 4.4). At Mt Bruce Wildlife centre *A. fumigatus* densities ranged from zero to 15,000,000 $CFUg^{-1}$, with total mean density of 1,500,000 ± 574,000 $CFUg^{-1}$. Densities recorded from the Massey University Campus ranged from zero to 19,000,000 $CUFg^{-1}$, with a total mean density of 2,900,000 ± 665,000 $CFUg^{-1}$. Both mainland sites have mean densities of *A. fumigatus* significantly higher (P < 0.02) than any of the island study sites. While the two mainland sites have similar ranges, mean densities were significantly higher at Massey University (P = 0.04). Mt Bruce also had a higher proportion of zero plate counts (38%) than Massey University (5%), and a lower proportion of counts above one million moulds per gram of soil (18%) compared to Massey University (28%). Therefore, while the spectrum of mould densities are similar at both study sites their distributions seem to be different. Mt Bruce seems to have a more patchy distribution of *A. fumigatus* moulds in the surface soil compared to the Massey University Campus study sites.

Aspergillus fumigatus densities from surface soils on Mokoia were more variable in edge and summit locations compared to interior forest locations. Densities of *A. fumigatus* in edge soils were about 4 times higher on Mokoia compared to LBI (Figure 4.5). However, some caution is required in comparing these results as data were collected during different years. Fungal counts from forest interior locations on Mokoia were about 9 times higher compared to LBI, and fungal counts from summit locations were about 20 times higher on Mokoia compared to LBI. On Mokoia, the summit areas seems to have similar *A. fumigatus* densities to the edge locations, while on LBI the
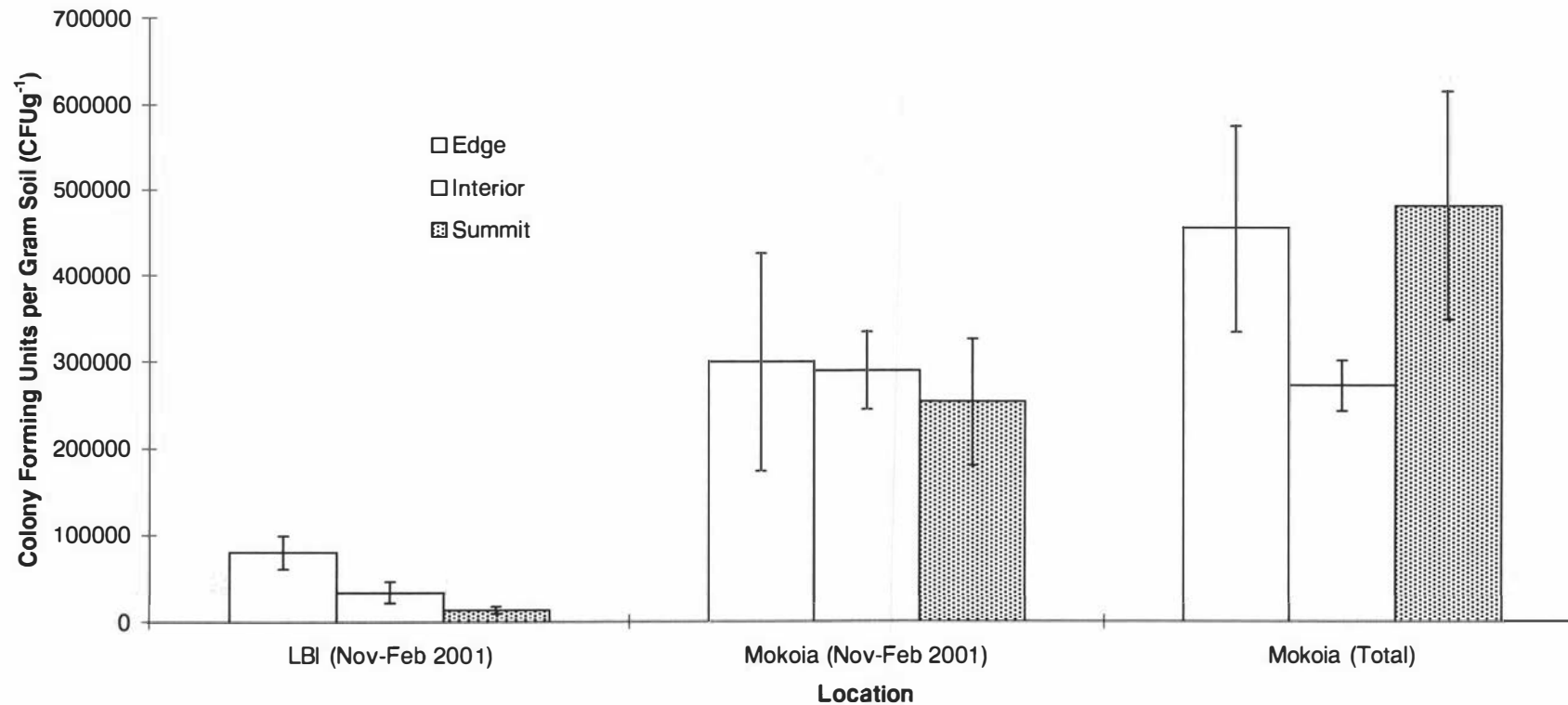
Figure 4.5. *Aspergillus fumigatus* densites from forest edge, interior and summit locations on Little Barrier and Mokoia Islands. Bars indicate standard error.

summit areas have similar densities to interior locations. At both study sites, edge areas tend to have elevated *A. fumigatus* densities compared to interior forest areas.

B) Flowers

In total, I collected 137 *Fuchsia* flowers during late July and August 2000 on Mokoia. *Aspergillus fumigatus* colony counts ranged from 0 to 700 CFU per flower, with a mean density of 137 ± 11 CFU per flower. From these samples, thirty eight *Fuchsia* flowers were collected from edge locations, and 81 flowers from interior locations on Mokoia. *Fuchsia* flowers collected from edge areas had densities that ranged from 100 to 700 CFU per flower, with a mean density of 264 ± 22 CFU per flower. *Fuchsia* flowers collected from interior locations had *A. fumigatus* densities ranging from 25 to 550 CFU per flower, with a mean density of 96 ± 11 CFU per flower.

I collected 66 Kohuhu flowers during September and October 2000. Colony counts ranged from 25 to 2600 CFU per flower, with a total mean *A. fumigatus* density of 395 ± 65 CFU per flower (Figure 6). I collected 33 kohuhu flowers from edge locations and these ranged from 100 to 2600 CFU per flower with a mean density of 675 ± 109 CFU per flowers. Kohuhu flowers collected from interior locations ranged from 0 to 250 CFU per flower, with a mean density of 98 ± 13 CFU per flower.

Sixty hangehange flowers were collected and screened for *A. Fumigatus* during August and September 2000. Colony counts ranged from 0 to 250 moulds per flower, with a mean density of 85 ± 8 CFU per flower. Thirty hangehange flowers were collected from edge and interior locations on Mokoia. Edge flower densities ranged from 0 to 205 CFU per flower, and interior flower densities ranged from 0 to 250 CFU per flower. Mean densities of *A. fumigatus* for edge flowers were 94 ± 11 CFU per flower, and interior flowers had a mean density of 77 ± 11 CFU per flower.

From forest edge and interior locations I collected 108 five-finger flowers during August and September 2000. *Aspergillus fumigatus* colony counts ranged from 0 to 375 CFU per flower, with a total mean density of 107 ± 8 CFU per flower. I collected 48 five-finger flowers from edge locations and 60 flowers from interior locations on Mokoia. Both sets of data had similar ranges (i.e., 0 to 250 CFU per flower). Edge
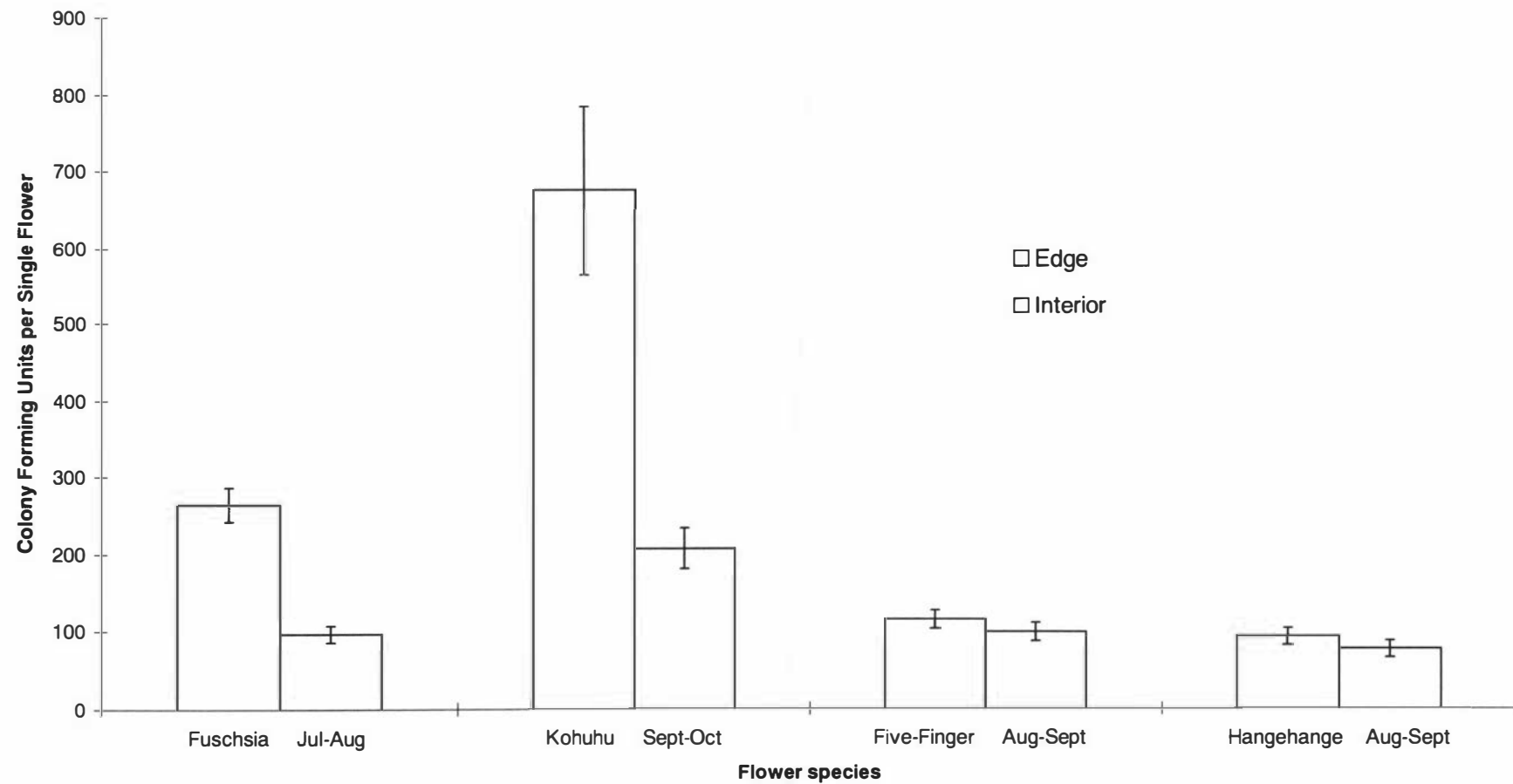
Figure 4.6. Mean *Aspergillus fumigatus* spore densities from flowers collected within 30 m of forest edge *vs* flowers collected from forest interior sites on Mokoia Island. Bars indicate standard error.
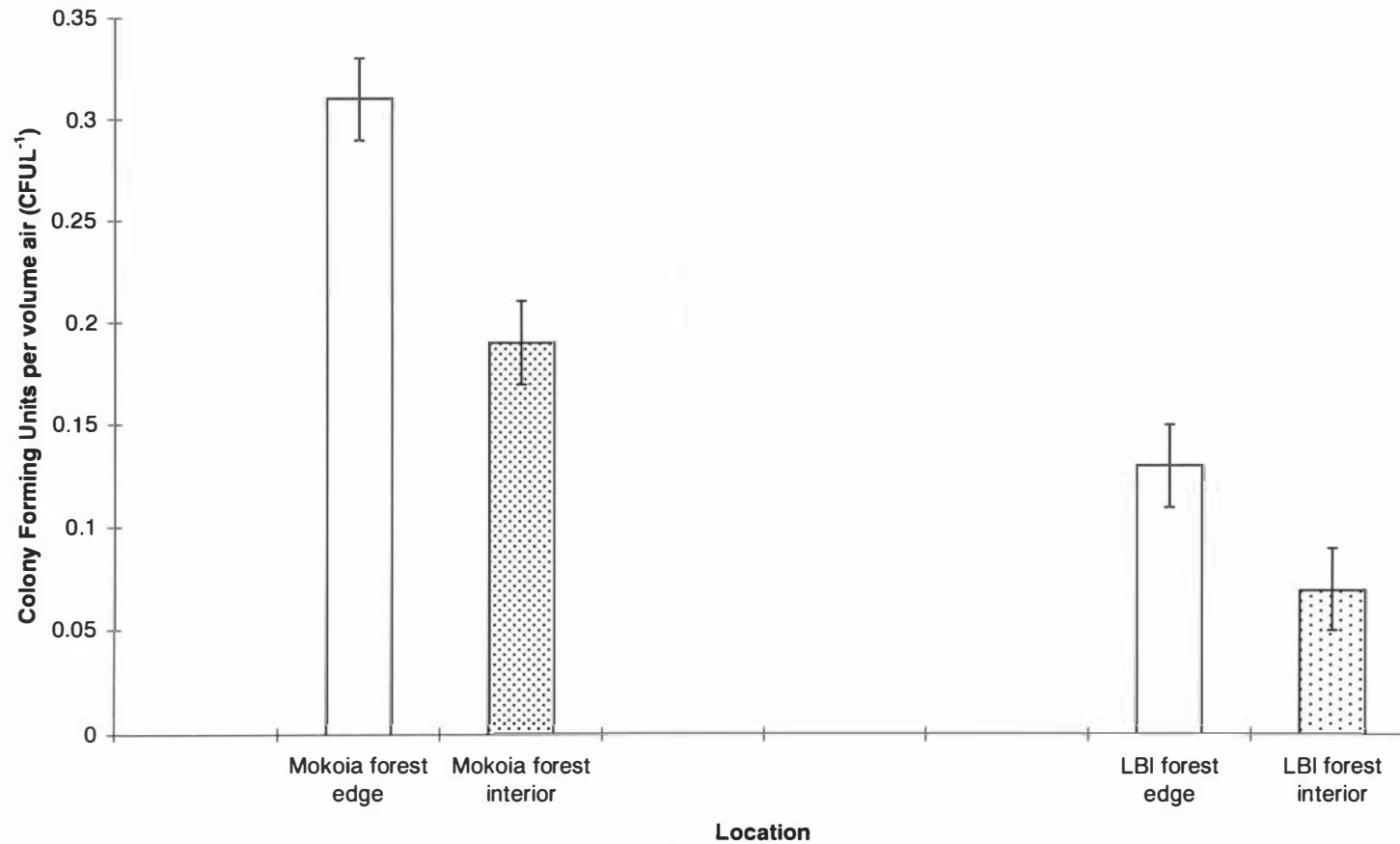
Figure 4.7. Densities of airborne *Aspergillus fumigatus* spores per volume of air (CFUL^{-1}) from forest edge and interior locations on Mokoia and Little Barrier Islands. Bars indicate standard error.

flowers had a mean density of 116 ± 12 CFU per flower, and interior flowers had a mean density of 99 ± 12 CFU per flower.

I found that edge flowers on average had higher mean *A. fumigatus* densities compared to interior flowers. I also found that *A. fumigatus* densities from interior flowers were generally more variable compared to those from edge flowers. However, significant differences between edge *vs* interior locations were found only for *Fuchsia* (P < 0.01) and kohuhu flowers (P < 0.01). While the mean *A. fumigatus* densities were also higher for edge *vs* interior locations for five-finger and hangehange flowers, these differences were not found to be significant (P = 0.2 and P = 0.1 respectively).

C) Air

The density of airborne *A. fumigatus* spores on Mokoia ranged from 0 to 0.73 CFU per litre of air (CFUL^{-1}), with a total mean density of 0.25 ± 0.02 CFUL^{-1}. Air samples collected along the forest edge averaged 0.31 ± 0.02 CFUL^{-1}, while forest interior samples averaged 0.21 ± 0.02 CFUL^{-1}(P <0.01). On LBI, the density of airborne *A. fumigatus* spores ranged from 0 to 0.9 CFU/m^3, with a total mean density of 0.1 ± 0.02 CFUL^{-1}. Air samples collected from forest edge habitat averaged 0.13 ± 0.02 CFUL^{-1}, while forest interior samples averaged 0.07 ± 0.02 CFUL^{-1}.

Airborne spore densities were about four times higher on Mokoia compared to LBI (Figure 4.7). Additionally, airborne spore densities were found to be significantly higher in edge *vs* interior locations for both Mokoia and LBI (P = 0.03).

Discussion

I found that the two mainland study sites had the highest mean *A. fumigatus* density estimates in surface soils, followed by Mokoia, whereas Tiritiri Matangi and LBI had the lowest estimates. This pattern may suggest that the closer you are to the mainland the higher the levels of *A. fumigatus*. The magnitude of the difference in *A. fumigatus* densities between the mainland sites and the furthermost island LBI are striking. Both mainland sites average about 2,200,000 ± 620,000 CFUg^{-1}, whereas, *A. fumigatus* densities average about 53,000 ± 23,000 CFUg^{-1} on LBI. This represents a 42 fold

difference in *A. fumigatus* densities between the two study sites. Densities of *A. fumigatus* on Tiri were the second lowest in the study (i.e., 151,000 ± 23,000 CFUg^{-1}), followed by Mokoia (i.e., 445,000 ± 35,000 CFUg^{-1}).

While both Mokoia and Tiritiri Matangi have received translocated hihi populations and share similar disturbance histories, they have several differences. Mokoia is the smallest of the island study sites (135 ha), the only island situated in a fresh water lake (Lake Rotorua), and the only island that is still geothermally active. Both Tiritiri Matangi and LBI are situated off the east coast of the upper North Island, and LBI followed by Tiritiri Matangi are the northern most study sites. LBI is the only study site that has not been previously clear-felled, and represents the last remaining vestige of virgin lowland forest in New Zealand. LBI is also home to the last remaining self-sustained population of Hihi, which until the late 1870s were widespread across the North Island (Oliver 1955).

Mokoia and Tiritiri Matangi were both occupied by Maori for hundreds of years prior to the arrival of Europeans. While both islands have had different regeneration histories, they were both clear-felled and converted to farmland last century, and the forests on both islands have been regenerating for about 40 to 50 years. However, the estimated *A. fumigatus* densities on Mokoia are about three fold higher compared to Tiritiri Matangi. Is it possible that forest disturbance regimes on Mokoia are more pronounced than they are on Tiritiri Matangi? For a start, Mokoia is technically a mainland site, situated in a small land-trapped lake (closest distance to the mainland is 2.1 km). Tiritiri Matangi on the other hand is a slightly larger offshore island situated about 4 km distance from the mainland. It is possible that in some locations on Mokoia soil temperatures vary unpredictably in response to fluctuations in thermal activity. Additionally, *Aspergillus* is well known for its ability to utilise sulphur (Wainwright 1988), which is, judging by the rotten-egg smell on Mokoia, not in short supply. Given the smaller size of the island, the youth of the vegetation, the unpredictable thermal activity, elevated sulphur levels, and proximity to the mainland, it may be possible that Mokoia is more exposed to natural and unnatural disturbance events and resource flushes compared to Tiritiri Matangi. One natural type of habitat variation that occurs predicably on Mokoia is the drying out of the leaf litter and surface soils over summer (Chapter 2). This annual habitat variation may contribute to elevated *A. fumigatus* levels on Mokoia and edge habitats by reducing

competition for resources on the forest floor. Because soil temperatures and water activities are less variable in old growth forests compared to regenerating forests, it is likely that the desiccation of the forest floor occurs less frequently in old growth forests.

In Chapter Two, I found evidence that *A. fumigatus* densities were high on Mokoia Island. This suggests that hihi might be suffering high levels of exposure to *A. fumigatus* spores on Mokoia. However, this does not lessen the importance of other potential primary causal factors, such as poor food supply and stress, which can reduce an individuals ability to fight disease. For example, free-living black swans in New Zealand have been reported suffering elevated aspergillosis infection rates when population numbers were above normal and the feed supply reduced (Williams 1964). Resistance to aspergillosis is seldom a problem for healthy birds. However, some bird populations, such as hihi on Mokoia, may be more exposed to *A. fumigatus* spores than other populations, i.e., hihi on LBI. If susceptibility to opportunistic infections is greater in sick, young and/or aged individuals, then populations composed predominantly of either will be at greater risk of infection compared to populations with normal age distributions. Additionally, small animals are more susceptible to starvation during lean periods compared to larger animals. Greater thermoregulatory costs incurred by small birds during egg brooding may reduce their health and constrain time spent warming eggs and/or feeding chicks. Living on a narrow margin between costs and benefits may predispose some wildlife populations and species to regular periods of poor health.

In terms of the original objectives, this study has shown there are significant differences in the densities of *A. fumigatus* between study sites, and between forest edge and forest interior locations. I found evidence that sites with relatively young, regenerating forests tend to have significantly higher levels of *A. fumigatus* compared to old growth forest sites. I also found significantly higher levels of *A. fumigatus* for forest edge habitats compared to inner forest locations, and this result held for air samples and flower samples (2 of 4 species) as well as surface soil samples. Experiments using flowers to test for *A. fumigatus* forest edge effects provided results that suggest that hihi on Mokoia are likely consuming *A. fumigatus* spores during nectar feeding. This may require us to take a closer look at the mycotoxin levels present in nectar on Mokoia, and whether hihi consumption of these toxins are such, that they are sufficient to effect their immunocompetence. Additionally, results indicate that that size of the flower and levels

of rainfall can have significant impacts on the levels of associated fungi. Flowers are easily collected and screened for fungi compared to soil. Because air sampling devices are problematic to use in the field and very expensive to buy, sampling flowers for fungal spores may be a useful method for indirectly estimating airborne spore levels.

The variable micro-climate within a young growth regenerating forest habitat is similar to the micro-climate found along the forest edge of an old growth forest. The greater stability of the inner-forest microclimate in old growth forests may partly explain their high microbial biodiversity and lower levels of *A. fumigatus*, i.e., fewer opportunities for invasion and establishment. In addition, birds like Hihi are forced to forage closer to the forest floor in young growth forests like Mokoia compared to old growth forests like LBI. This situation may further increase their exposure to *A. fumigatus* in young growth forests. Dry winds damage vegetation, creating tree falls on the forest edge and tree fall gaps in the forest interior (Lovejoy *et al* 1986). Laurence *et al.* (1998), in a study examining fragmentation effects on Amazonian tree communities, suggest that a sudden increase in gap-phase vegetation could help drive local extinctions of disturbance-sensitive species (i.e., *K*-strategists) in fragments. By opening up the forest floor to more light and as a result to higher soil temperatures, tree fall gaps can prevent the regeneration of shade-tolerant, moisture-adapted organisms, and instead promote the establishment of shade-intolerant organisms that do not require moist soils. Species in small or thin fragments, with higher edge to interior ratios, are particularly vulnerable to the effects of wind damage because wind has the potential to penetrate deep into a forest. Due to this, small fragments will usually have a higher proportion of their area in gaps (Laurence *et al.* 1998). These physical edge effects have been shown to increase plant growth rates, elevate rates of mortality, reduce stocking density, and differentially affect regeneration of native *vs* exotic plant species (Chen *et al.* 1992).

Previous studies indicate that replacement of forest with grassland causes an alteration of the soil food-web structure from a fungal-dominated food-web in forests to a bacterial-dominated food-web in grasslands (Ingham *et al.* 1986 a, b; 1991; Ingham and Thies 1995). The processes of immobilisation and mineralisation are tightly coupled to plant growth in undisturbed ecosystems, this coupling may be lost or reduced following disturbance (Ingham and Coleman 1984; Hendrix *et al.* 1986; Nannipieri *et al.* 1990). Increasing complexity of the soil food-web in old growth ecosystems reduces nutrient

loss from the system. The greater the food-web complexity, the greater the retention of nutrients within the soil (Coleman *et al.* 1992). With this in mind, it is likely that habitat disturbance opens resource reserves to opportunistic organisms such as *A. fumigatus*. This in turn implies that biologically diverse ecosystems, such as those found in old growth forests, potentially function in keeping weedy pest species like *A. fumigatus* in check.

Are species-specific generalisations all we can hope for regarding changing species assemblages with disturbance and succession? Or are there statements we can make about changes in species functional groups as a result of disturbance? It is well known that old growth forests play an important role in the maintenance of global weather patterns and the diversity of the earths biota (e.g., Walter 1985; Woodward 1987; Wilson and Peter 1988; Leemans *et al.* 1995). As mentioned, the creation of complex soil food-webs found in old growth forests potentially play an important role in regulating the abundance and distributions of opportunistic pathological microbes like *A. fumigatus*. Invasion and colonisation by weed-like cosmopolitan species are disturbance driven, but also disturbances in their own right. These invaders reduce the health and integrity of regenerating ecosystems in many ways, and can lead to endangerment and extinction of native species (Pickett and White 1985; Pementel 1988). Generally, it is apparent that the influence of habitat disturbance on any species will be determined largely by that organisms environmental tolerances, mobility, and ability to find shelter. It is doubtful whether generalisations can be made about the effects of habitat disturbance without detailed site-specific and species-specific studies.

There is little doubt that fragmentation-induced changes in abundance and species richness occur in many soil fungi groups. However, the study of soil fungi in fragmented forests is still in its infancy and is data poor. If there exists a link between forest edge effects and the abundance of *Aspergillus*, then it is possible that habitat modification and fragmentation predisposes some bird species, like hihi, to diseases that they historically had little contact with. This in turn may predispose birds to starvation and/or predation pressure in young growth forests and forest edge habitats.

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Chapter 5

Aspergillus fumigatus densities in Hihi cavity nests on Mokoia and Little Barrier Islands

Introduction

During my field work in 1998 I collected cicada remains infected with *A. fumigatus* inside and outside active hihi nest boxes on Mokoia Island. Hihi rear their chicks on insects including cicadas, and it seems likely that they are transporting this fungus back to their nest boxes while carrying out their parental duties. After finding a potential link between *A. fumigatus* and the hihi food supply and nesting environment, I decided to measure the levels of *A. fumigatus* in hihi nest boxes on Mokoia and natural tree cavity nests on Little Barrier Island (LBI). To investigate whether nest boxes on Mokoia were encouraging the growth of *A. fumigatus* I decided to measure air temperature and relative humidity (RH) within hihi nest boxes on Mokoia and tree cavity-nests on LBI. Therefore, this chapter is directed towards identifying whether nest box placement and conditions are encouraging the growth of *A. fumigatus*, and whether nest boxes are a likely source of infection to hihi on Mokoia Island.

While hihi readily use artificial nest boxes on Mokoia, they are also prone to abandoning these sites during the early stages of nest building or incubation (*pers. obs.*). Conditions leading to nest abandonment in hihi are unknown. However, there is concern that this may be a result of deficiencies in either nest box design and/or placement. Nest abandonment requires birds to spent more time locating and rebuilding new nesting sites, and this may disrupt breeding activities and cause breeding females psychological stress. Although hihi will use nest boxes, this does not necessarily demonstrate their suitability. Factors related to nest box placement such as local microclimatic factors and food availability might be more important than nest box design. For instance, Castro *et al.* (In press) studied the effects of food supplementation on nest box use by hihi on

Mokoia, and found that food supplementation resulted in changes in hihi incubation behaviour. Females at fed nest boxes spent less time in the nest box and less time foraging on natural food supplies. While incubation times were shorter at fed nests (15.6 *vs.* 16.4 days), individual incubation bouts tended to last longer (16%). If nest boxes are an important source of disease to hihi, then supplement feeding reduces the overall time females and chicks spend in the nest box. This in turn may reduce levels of exposure to *A. fumigatus* experienced by hihi on Mokoia. Alternatively, supplement feeding may allow females to better time-budget disease prevention behaviours such as personal hygiene and sunning activities. Therefore, while supplement feeding probably reduces overall levels of hunger and improves disease resistance among hihi, this may be primarily related to increased time given to grooming and sunning activities. These disease prevention behaviours should be especially important to birds suffering poor health and/or elevated exposure to disease-causing agents.

Tieke, or saddlebacks (*Philesturnus carunculatus*) were translocated to Mokoia Island during 1992, and unlike hihi, have survived well on the island with nest boxes provided. Saddlebacks are highly territorial birds during the breeding season, and their territories on Mokoia are usually a combination of gully, slope and ridge type habitats. While saddlebacks regularly forage for insects and fruits in the gully and slope areas they tend to nest predominantly in the dry scrubby ridge locations. In contrast, hihi on Mokoia nest predominately in gully areas and seem to avoid nest boxes in ridge locations (D.P. Armstrong- *pers. comm.*). Is it possible that the placement of the nest box influences the levels of *A. fumigatus* within the nest box? By nesting in gully habitats are hihi nest boxes more prone to high densities of *A. fumigatus*? There is tentative evidence that suggests this is true as *A. fumigatus* densities in the soil were two-fold higher in gully locations compared to ridge locations during the breeding season on Mokoia (see Chapter 3). Therefore, in this chapter I will address the question of whether nest box *A. fumigatus* densities can be related to nest box placement on Mokoia.

Why hihi choose one nest box over another is a difficult question to answer without more detailed information on natural cavity nesting by hihi. Very little research has been done on natural cavity nesting in hihi on LBI. Angehr (1984a,b, 1985) and Rasch (1985a,b; 1989) are the only studies to investigate cavity nesting in hihi on LBI. They demonstrated that hihi will use artificial nest boxes, and that hihi do not reuse the same

tree-cavity nest for their second clutch on LBI. Birds re-using old nesting material could suffer increased exposure to microbial pathogens (Rendell and Verbeek 1996; Singleton and Harper 1998). Therefore, hihi unwillingness to re-use old nest sites may be a type of disease avoidance that allows birds to reduce their exposure to disease-causing organisms such as *A. fumigatus*. Rasch (1989) concluded that hihi reproduction on LBI was not constrained by the availability of suitable tree cavities. However, Castro (1994a,b; 1995) found evidence that this is not true for hihi on Kapiti and Mokoia Islands with artificial nest boxes provided. Castro (1994a,b; 1995) found that hihi tended to re-use cavities for their second clutch. Castro (1995) suggested that this was probably the result of intense competition for nest sites, which could be reflecting limited availability of suitable cavities on Kapiti Island. Hihi subsequently translocated to Mokoia and Tiritiri Matangi Islands were provided with an abundance of artificial nest boxes, and had done most of their nesting in these boxes.

There are many examples regarding cavity nesting birds, where the addition of artificial nest boxes have increased the number of breeding pairs in the area (e.g, von Haartman 1957; Brush 1983; Nilsson 1984; Willner et al. 1983; Johnson and Kermott 1994; Ingold 1997; Gunn and Hagan 1999), and this suggests that natural tree-cavities are limited in many forest habitats. This may indicate that Rasch (1989) did not get an increase in mating pairs in her treatment areas because the nest boxes she provided may have been unsuitable to most hihi on LBI. The purpose of this chapter is to investigate the microclimate inside and outside active hihi nest boxes on Mokoia, and puriri tree cavity-nests on LBI. I will describe the microclimates between internal *vs* external nest cavity locations, and their associated levels of *A. fumigatus*. These results are then compared to those in Chapter Four to give an indication as to whether hihi nest boxes on Mokoia are a likely source of aspergillosis. The primary question this chapter addresses is whether cavity-nesting behaviour in hihi is likely to increase their exposure to *A. fumigatus* when using artificial nest boxes. This is important because, as pointed out in Chapter Four, hihi may have had historically little contact with *A. fumigatus*, and therefore exhibit low immunity to *A. fumigatus* infections.

This chapter reports work designed to achieve the following objectives, 1) Compare *A. fumigatus* densities recorded from old nesting material to fresh nesting material 2) determine whether placement of the nest box influences the levels of *A. fumigatus* in the

nesting material 2) Compare *A. fumigatus* densities estimates recorded from hihi nest boxes to *A. fumigatus* densities recorded from natural tree-cavity nests on LBI 3) Determine whether the microclimate within the nest box encourages the growth of *A. fumigatus* on Mokoia Island.

Methods

1) *Location of nesting sites and placement of dataloggers*

On Mokoia Island, all active nest boxes were attached to mahoe trees about four to five feet above the ground (Figure 5.1). Most nest boxes, other than those at the Hotpool, Totara Grove and Cliff Richard sites were in gully locations under a seral mahoe canopy, i.e., K-Gully, D-Gully, Upper Queen St Gully, and Lower Queen St Gully. From November 1999 to January 2000 Hobo dataloggers were set up inside nest boxes that hihi were building nests in. These internal dataloggers were attached to the roof of each active nest box and allowed to hang-down into the nesting cavity about 15 to 20 cm above the nest-cup. They were set up during nest building to ensure hihi would continue to build their nests with dataloggers present. Each internal datalogger was accompanied by an external datalogger placed outside the nest box on the opposite side of the tree. External datalogger were attached to the inside of an ice-cream container to keep the datalogger dry. The up-turned ice-cream container was then screwed to the tree and at the same height as the nest box. If a nest box was abandoned early by hihi the internal datalogger was removed until the location of another active nesting site was known, then the procedure was repeated.

The dataloggers recorded air temperature and relative hourly inside and outside every active nest box in the study. Mean air temperature and RH were averaged daily, and averaged each month. Maximum and minimum mean air temperature and relative humidity were also recorded daily, and averaged each month to give a monthly mean maximum and monthly mean minimum for both air temperature and relative humidity.

On LBI, dataloggers were set up and substrate samples collected from five natural nest cavities in puriri trees (*Vitex lucens*). This was done to compare *A. fumigatus* levels and conditions within a natural tree cavity to those outside the tree cavity. I initially aimed
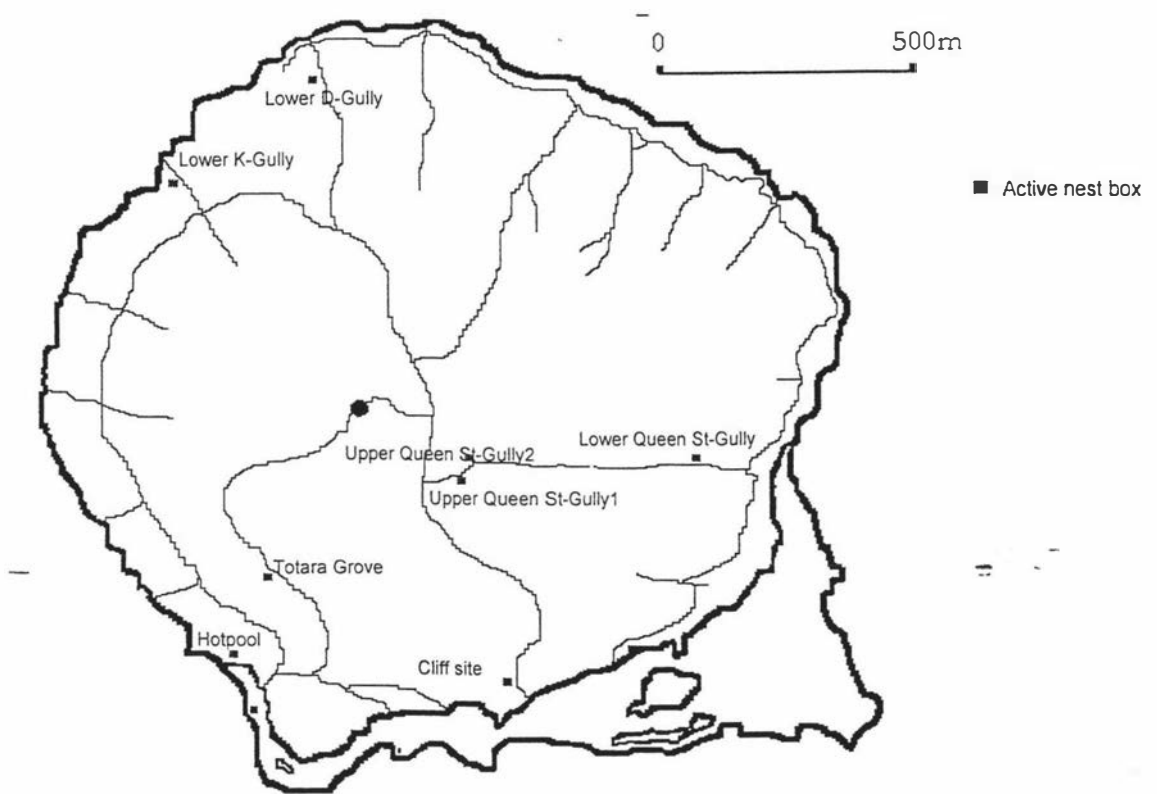
Figure 5.1. Map of Mokoia showing location of active hihi nest boxes during the 1999/2000 breeding season

to compare data collected from active hihi nest boxes on Mokoia to data collected from active hihi cavity-nests on LBI. Because I failed to find any active hihi nest cavities on LBI, dataloggers were set up and substrate samples taken from cavity-nests being used by other bird species. Because of this, results from the two islands are not directly comparable.

On LBI I found five cavity nests, all in puriri trees. I found one partially complete hihi nest in a tree trunk cavity about 200m up from the mouth of the Tirikakawa Stream on the east bank about 30 m up the gully. I found one active morepork (*Ninox n. novaeseelandiae*) cavity nest in a tree branch about 500 m up the Waipawa Stream located on the west bank about 20 m up the gully. Near the morepork nest I found two saddleback cavity nests and one kaka nest under construction in three separate tree branches. At each cavity nest I set up two dataloggers (i.e., one internal and one external) in the same manner described for nest boxes on Mokoia. In total 10 dataloggers were set up at the five sites on LBI during November 2000, and all were removed during May 2001.

2) *Collection of nesting material, soil, potential nesting material and air*
Substrate samples collected include, 1) internal nesting material from Hihi nest boxes on Mokoia, and from tree cavity nests on LBI, 2) external surface soil samples collected within 20 m of each nest site, 3) internal air samples collected from four nest boxes on Mokoia using a SAS spore impacter, and 4) potential nesting material, i.e., dry twigs, fern hairs, dry grasses, moss and lichens collected from nesting locations on Mokoia and LBI.

Nesting material was collected from eight hihi nest boxes by pulling material through the entrance hole using forceps, and placing it in a sterile container. Nesting materials were collected when the breeding females were absent from the area to prevent abandonment of the nest. Potential nesting material was collected at each nesting location at the same time as the actual nesting material. Potential nesting material consisted of items commonly used in hihi nests such as dry twigs, fern hairs, dry grasses, and lichen. On LBI the same technique was used to collect nesting material from five cavity nests, mentioned above. The whole nest was removed at this stage and transported to the laboratory for processing.

Soil samples were taken at 5 m intervals along 20 m transect lines starting at the nesting site following a specific compass bearing. Soil samples were collected by scraping a sterile container across the ground until about 50 g of soil was collected to a depth of two to three centimetres.

Potential nesting materials included an assortment of twigs, fern hairs, dry grasses, and lichens of similar dimensions to those found in actual hihi nests, and were collected near active nest boxes. These materials were collected on Mokoia in October and November 1999, and on LBI in late November and early December 2000. In total, I screened 40 X 1g potential nesting material substrate samples for *A. fumigatus* on Mokoia. Seven of the eight active nest boxes were provisioned by hihi with nesting material during early October 1999. Potential and actual nesting materials were collected by hand while wearing surgical gloves and placed in sterile containers for transport back to the laboratory.

Air samples were collected from five of the eight nest boxes shortly after the last chick had fledged or died. Nest box air samples were collected from Hotpool, Totara Grove, Lower K-gully, Upper Queen St-gully1, and Lower Queen St-gully. Air samples were collected onto standard PDA medium using a calibrated Surface Air System™ (SAS) single-stage sieve (219 jet) culture plate impactor. Air samples were collected by placing the SAS spore impacter up against the entrance to the nest box so air could be sucked out through the nest box. Each air sample took 20 seconds to impact 60 litres of air onto a PDA plate, which was then sealed with tape and transported back to the laboratory for incubation. Samples were incubated for three days at 38^0C (see Laboratory methods).

3) *Laboratory methods*

The number of *A. fumigatus* propagules per gram of dry substrate was estimated using the dilution plate technique (Johnson et al. 1959). Moisture content of each soil and leaf litter sample was estimated by removing 1g of wet soil and drying this in an oven at 37^0C until constant weight was achieved (2 to 3 days). This was then functioned into the final plate counts to estimate the number of fungal colonies per gram dry soil and leaf litter. Ten grams of wet substrate was then mixed with one litre of sterile distilled water

and blended at high speed for about three minutes. Three stock solutions of 1:100 were then prepared for each substrate. From these stock solutions three serial dilutions of 1:1000 and 1:10000 and 1:100000 were prepared. One of the defects in the dilution plate technique is that during the transference of soil suspension by pipetting, heavy soil particles settle down quickly taking with them the suspended fungal propagules and this affects greatly the final count. To reduce this I added 0.05 ml of Tween 20 (suspension agent) per 100ml of soil suspension.

Fungal colony forming units (CFUs) were estimated by pipetting and spreading 0.2 ml of each dilution onto standard potato-dextrose agar (PDA) media with 30 mg of chloramphenicol per litre agar added. Three plates were used for each dilution, and were incubated at 38^0C for 4 days during which they were examined daily. The average CFU count was multiplied by the dilution factor to estimate the number of CFUs per gram of soil for each plate count. A set of blanks were also run for each batch of sample plates by inoculating three PDA plates with sterile distilled water instead of soil suspension. On these no fungi developed while liberal growth had occurred on the soil inoculated plates; only in some cases mycelium appeared on the very edge of the plates. Additionally, to standardise the procedure aliquots were taken from the centre of the suspension.

Identifications of fungal colonies grown on culture plates were made using the diagnostic keys for aspergilli and fungi imperfecti from monographs by Raper and Fennell (1973), Subramanian (1971), and Latge (1995). *Aspergillus fumigatus* colonies were identified on the basis of colony morphology and spore formation and size.

Results

1) Aspergillus fumigatus *densities from active nest boxes on Mokoia Island*

Mean *A. fumigatus* densities recorded from inside active hihi nest boxes on Mokoia during the 1999/2000 breeding season ranged from $113,958 \pm 34,623$ CFUg^{-1} to $308,056 \pm 69,170$ CFUg^{-1} to, with an overall average of $195,651 \pm 26,769$ CFUg^{-1} (Table 5.1; Figure 5.2). *Aspergillus fumigatus* densities recorded outside hihi nest boxes ranged from $103,750 \pm 23,281$ CFUg^{-1} to $1,067,917 \pm 208,409$ CFUg^{-1}, with an overall mean of

Mokola Internal Location	Nesting period	Nestbox CFUg^{-1}	St. Error CFUg^{-1}	Mean Tot. Int. Air Temp	Mean Max. Int. Air Temp	Mean Min Int. Air Temp	Mean Tot. Int. RH	Mean Max. Int. RH	Mean Min Int. RH
Hotpool	Oct-Dec	3.03E+05	8.11E+04	17.8	22.6	16.2	91.4	96.1	86.5
Totara Gr	Jan	3.08E+05	6.92E+04	17.1	19.7	14.9	87.7	90.8	83.6
LKG	Oct-Nov	1.14E+05	3.46E+04	16.2	20.3	15.3	94.6	97.2	91.3
LDG	Nov-Jan	1.28E+05	3.48E+04	17.3	19.5	14.5	88.3	92.5	86.3
UQSG1	Oct-Jan	1.70E+05	5.11E+04	16.3	19.8	13.4	90.4	92.3	85.1
UQSG2	Oct-Dec	2.39E+05	4.74E+04	16.1	19.2	13.5	88.2	94.4	87.0
LQSG	Nov-Mar	2.04E+05	3.40E+04	17.7	21.2	15.9	92.2	95.2	86.5
CLIFF	Oct-Nov	1.48E+05	3.24E+04	17.6	19.6	14.4	91.5	92.6	85.1
Total mean		**1.96E+05**	**2.68E+04**	**17.0**	**20.2**	**14.8**	**90.5**	**93.9**	**86.4**

Mokola External Location	Nesting period	Soil CFUg^{-1}	St. Error CFUg^{-1}	Mean Tot. Ext. Air Temp	Mean Max. Ext. Air Temp	Mean Min Ext. Air Temp	Mean Tot. Ext. RH	Mean Max. Ext. RH	Mean Min Ext. RH
Hotpool	Oct-Dec	2.00E+05	4.66E+04	13.1	18.9	11.6	88.0	96.4	79.8
Totara Gr	Jan	2.00E+05	4.98E+04	16.2	20.6	13.8	87.1	95.3	79.8
LowerK-Gully	Oct-Nov	1.07E+06	2.08E+05	12.6	18.0	11.0	81.6	97.7	79.8
Lower D-Gully	Nov-Jan	6.14E+05	1.53E+05	14.3	19.7	12.3	88.3	96.6	78.1
Upper Queen St Gully1	Oct-Jan	3.91E+05	1.08E+05	13.5	18.2	10.2	87.3	99.9	82.0
Upper Queen St Gully2	Oct-Dec	4.21E+05	1.22E+05	12.8	18.2	10.1	89.7	98.1	78.9
Lower Queen St Gully	Nov-Mar	1.04E+05	2.33E+04	14.7	18.2	12.0	89.3	97.4	78.5
CLIFF	Oct-Nov	3.80E+05	7.53E+04	14.4	18.5	11.4	87.5	96.6	78.1
Total mean		**4.01E+05**	**1.08E+05**	**14.0**	**18.8**	**11.6**	**87.3**	**97.2**	**79.4**

LBI Internal Location	Month	Cavity CFUg-1	St. Error CFUg-1	Mean Tot. Int. Air Temp	Mean Max. Int. Air Temp	Mean Min Int. Air Temp	Mean Tot. Int. RH	Mean Max. Int. RH	Mean Min Int. RH
SB1 crevice	Dec	6.67E+04	2.11E+04	17.1	17.8	15.7	82.0	89.6	80.0
Owl crevice	Dec	1.12E+05	2.57E+04	17.3	18.7	15.9	89.2	95.1	81.4
Hihi crevice	Dec	4.25E+04	1.42E+04	17.6	19.1	16.6	83.3	87.3	78.6
Kaka crevice	Dec	8.83E+04	1.81E+04	17.4	18.9	16.2	87.3	93.2	80.2
SB2 crevice	Mar	1.10E+05	4.25E+04	17.2	17.9	16.6	99.9	100.0	99.0
Total mean		**8.38E+04**	**1.34E+04**	**17.3**	**18.5**	**16.2**	**88.3**	**93.0**	**83.8**

LBI External Location	Month	Soil CFUg-1	St. Error CFUg-1	Mean Tot. Ext. Air Temp	Mean Max. Ext. Air Temp	Mean Min Ext. Air Temp	Mean Tot. Ext. RH	Mean Max. Ext. RH	Mean Min Ext. RH
SB1 crevice	Dec	3.31E+04	1.13E+04	17.5	20.5	15.1	83.7	94.6	67.7
Owl crevice	Dec	2.11E+03	7.10E+02	17.6	21.5	15.3	88.4	97.8	71.4
Hihi crevice	Dec	2.62E+04	5.91E+03	17.8	21.0	15.5	88.2	97.2	72.1
Kaka crevice	Dec	1.35E+04	5.32E+03	17.6	21.3	15.4	89.1	98.0	73.2
SB2 crevice	Mar	2.17E+04	5.91E+03	16.6	19.0	14.8	89.9	98.9	75.5
Total mean		**1.93E+04**	**5.44E+03**	**17.4**	**20.6**	**15.2**	**87.9**	**97.3**	**72.0**

Table 5.1. Summary of *Aspergillus fumigatus* densities and micro-climate results from inside and outside hihi nestboxes on Mokoia and inside/outside puriri tree cavity nests on Little Barrier Island
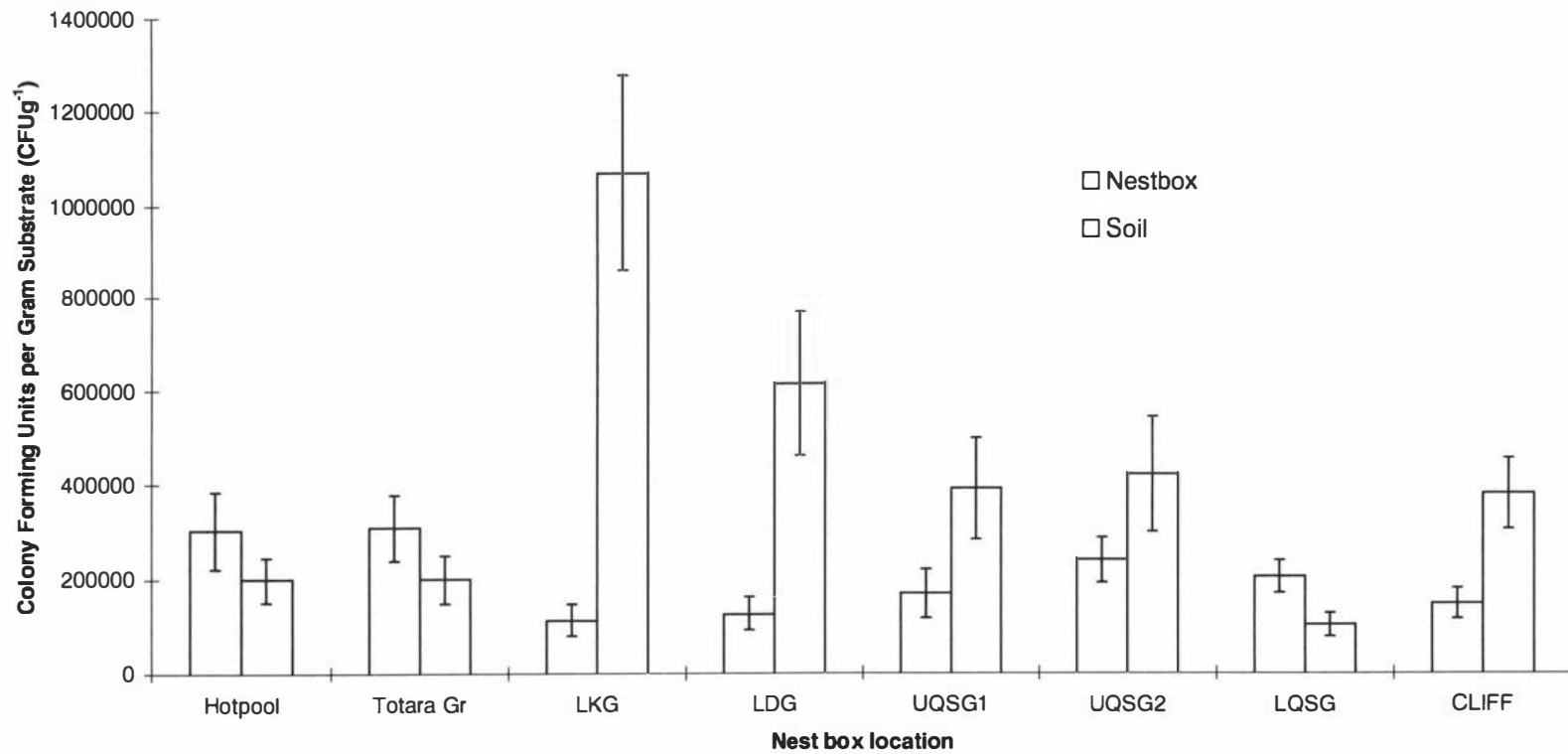
Figure 5.2. *Aspergillus fumigatus* density estimates for Hihi nest box material and surface soil collected from around active nest boxes on Mokoia Island. Bars indicate standard error.

400,819 ± 108,219 CFUg^{-1}. Therefore, on average *A. fumigatus* densities from active hihi nest boxes were about 34% below levels recorded from soil outside the nest boxes on Mokoia. This suggests that nest boxes are not encouraging the growth of *A. fumigatus* on Mokoia.

2) *Microclimate conditions inside and outside hihi nest boxes on Mokoia Island*

Monthly mean air temperatures within active hihi nest boxes on Mokoia ranged from 16.2^{0}C to 17.8^{0}C, with an overall mean air temperature of 17.0^{0}C (Table 5.1). The monthly mean maximum internal air temperatures ranged from 22.6^{0}C to 19.2^{0}C, with a maximum mean air temperature of 20.2^{0}C. Monthly mean minimum air temperatures ranged from 16.2^{0}C to 13.4^{0}C for all nest boxes, with an overall mean minimum air temperature of 14.8^{0}C. Mean RH inside active hihi nest boxes ranged from 94.6% to 88.2%, with an overall mean RH of 90.5%. Maximum mean RH estimations ranged from 97.2% to 90.84%, with an overall mean maximum RH of 93.9%. Mean minimum RH estimations ranged from 87.0% to 83.6%, with a mean minimum RH of 86.4%.

External dataloggers set up in association with active hihi nest boxes on Mokoia were analysed in the same manner as internal dataloggers. Mean external air temperatures ranged from 16.2^{0}C to 12.8^{0}C, with an overall mean external air temperature of 13.9^{0}C (Table 5.1). Mean maximum external air temperatures ranged from 20.6^{0}C to 18.0^{0}C, with an overall mean maximum air temperature of 18.78^{0}C. Mean minimum air temperatures ranged from 13.8^{0}C to 10.1^{0}C, with an overall minimum air temperature of 11.6^{0}C. Mean RH estimations ranged from 89.7% to 81.6%, with an overall mean RH for all sites of 87.3%. Mean maximum RH estimations ranged from 99.9% to 95.4%, with an overall mean maximum RH of 97.3%. Mean minimum RH estimations ranged from 82.0% to 78.1%, with an overall minimum RH of 79.4%. These datum indicate that internal air temperatures in active nest boxes were on average about 3^{0}C warmer compared to the mean air temperature outside the nest boxes. Mean air temperatures inside active nest boxes ranged 5.47^{0}C, while mean air temperatures outside nest boxes ranged 7.22^{0}C. Mean RH estimations within nest boxes tended to be 3.2% higher compared to mean RH estimations recorded outside hihi nest boxes. Mean RH estimations ranged 7.48% within active hihi nest boxes, while external RH estimations ranged 17.86%. Therefore, air temperatures and relative humidity levels are higher and

less variable within an active nest box internal nest box conditions are less variable compared to conditions external to the nest box.

3) *Proportion of water per gram of nesting material and soil on Mokoia Island*

On Mokoia the percent of water per gram of nesting material recorded during November 1999 ranged from 7 to 42 %, with an overall mean of 21.7 ± 2.5 % (Figure 5.3). External surface soil samples ranged 50 to 26 %, with an overall percent water content of 34.4 ± 2.5 % (see Chapter 3). These results indicate that the percent water content in the surface soil is about 1.6 times higher compared to nest box nesting materials on Mokoia. This may indicate that dry conditions within the nest box are preventing *A. fumigatus* from achieving similar densities recorded in the soil samples. This may imply that nest boxes situated in damp gullies are more likely to have higher *A. fumigatus* densities compared to nest boxes situated in drier ridge locations.

4) Aspergillus fumigatus *densities from natural cavity-nests on Little Barrier Island*

Mean *Aspergillus fumigatus* densities recorded from inside natural tree cavity-nests on LBI ranged from 111,667 ± 25,695 CFUg^{-1} to 42,500 ± 14,201 CFUg^{-1}, with a total average of 83,750 ± 13,371 CFUg^{-1} (Figure 4) Mean *A. fumigatus* densities recorded from outside tree cavities ranged from 26167 ± 5912 CFUg^{-1} to 2111 ± 710 CFUg^{-1}, with a total mean density of 19,306 ± 5441 CFUg^{-1}. Therefore, on average *A. fumigatus* densities from natural tree cavities were about 62% higher compared to surface soil samples collected from outside the tree cavity-nests. These results are in contrast to Mokoia, where external soil samples gave higher fungal counts compared to nesting material collected from nest boxes.

5) *Microclimate conditions inside and outside cavity-nests on Little Barrier Island*

Monthly mean air temperatures within tree cavities on LBI ranged from 17.6^{0}C to 17.1^{0}C, with an overall mean air temperature of 17.3^{0}C (Table 5.1). The mean maximum internal air temperatures ranged from 19.1^{0}C to 17.8^{0}C, with an overall maximum mean air temperature of 18.5^{0}C. Mean minimum air temperatures ranged from 16.6^{0}C to 15.7^{0}C for all tree cavities, with an overall mean minimum air temperature of 16.2^{0}C. Mean RH estimations recorded inside tree cavities on LBI ranged from 99.9% to 81.9%, with an overall mean RH of 88.3%. Maximum mean RH estimations ranged from 100%
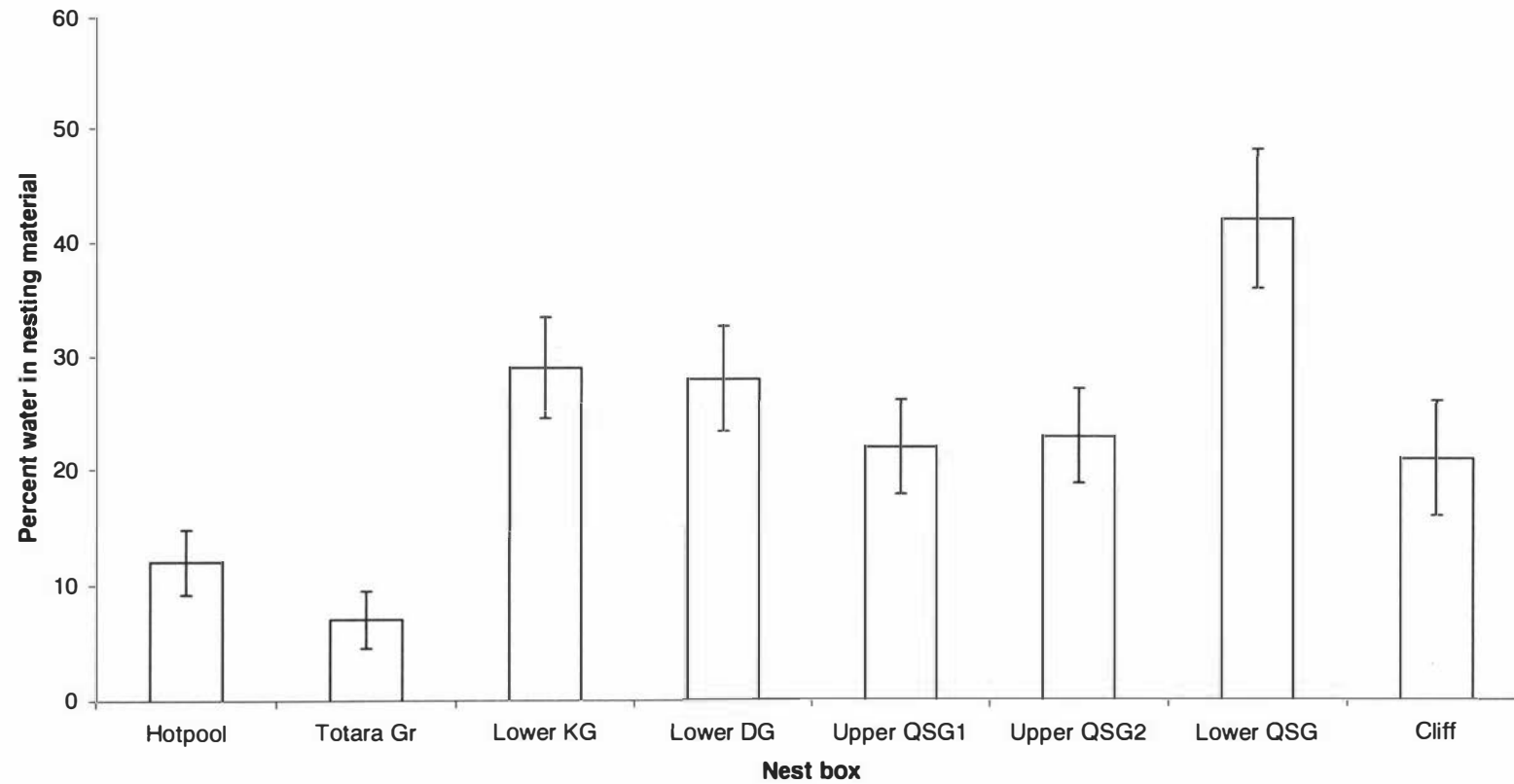
Figure 5.3. Variations in percent water per gram of nesting material collected from active nest boxes on Mokoia Island. Bars indicate standard error.

to 87.3%, with an overall mean maximum RH of 93.0%. Mean minimum RH estimations ranged from 99.0% to 80.2%, with an overall mean minimum RH of 83.8%.

Mean external air temperatures ranged from 17.8^0C to 16.6^0C, with an overall mean external air temperature of 17.4^0C (Table 5.1). Mean maximum external air temperatures ranged from 21.5^0C to 18.9^0C, with an overall mean maximum air temperature of 20.7^0C. Mean minimum air temperatures ranged from 15.52^0C to 14.81^0C, with an overall mean minimum air temperature of 15.2^0C. Mean RH estimations ranged from 89.9% to 83.7%, with an overall mean RH for all sites of 87.9%. Mean maximum RH estimations ranged from 98.9% to 84.6%, with an overall mean maximum RH of 97.3%. Mean minimum RH estimations ranged from 75.5% to 67.7%, with an overall mean minimum RH of 72.0%. These results indicate that mean air temperatures inside tree cavities ranged 2.3^0C, while mean air temperatures outside tree cavities ranged 5.4^0C. Mean RH estimations within tree cavities were similar to the mean RH estimations recorded outside the tree cavities. Mean RH estimations ranged 9.2% within the tree cavities, while external RH estimations ranged 22.6%. Therefore, while mean internal and external air temperatures and relative humidity levels are similar, the external environment exhibits greater variations in temperature and RH. The microclimate inside a tree cavity tends towards higher humidity and lower air temperatures compared to the external environment. External RH on LBI can drop as low as 72% (or 0.72 *aw*) at some locations, which is below the minimum water activity tolerance limit for *Aspergillus*. *Aspergillus fumigatus* densities were about four times higher in the natural tree cavity environment compared to the external surface soil environment.

6) *Proportion of water per gram of nesting material and soil on Little Barrier Island*
On LBI the proportion of water per nesting material recorded from gram of natural tree cavities during November 1999 ranged from 27 to 9%, with a combined mean percent water content of 16.3 ± 1.5% for all tree cavities (Figure 5.5). External surface soil samples ranged 50 to 20%, with a mean percent water content of 35.8 ± 1.1%. These results indicate that cavity nest material on LBI is about 2.2 times drier compared to the surface soil collected outside the cavity nest.
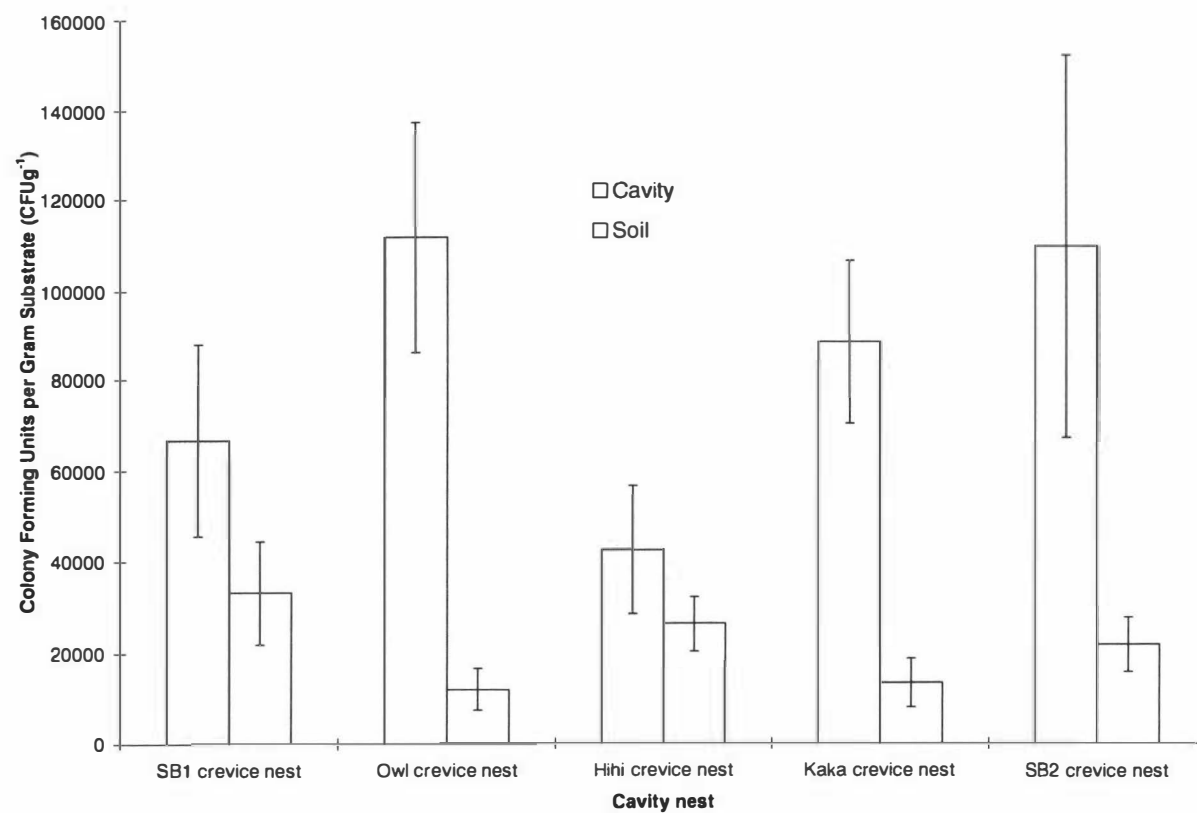
Figure 5.4. *Aspergillus fumigatus* densities from natural tree cavity-nests and soil samples collected around nesting areas on Little Barrier Island. bars indicate standard error.
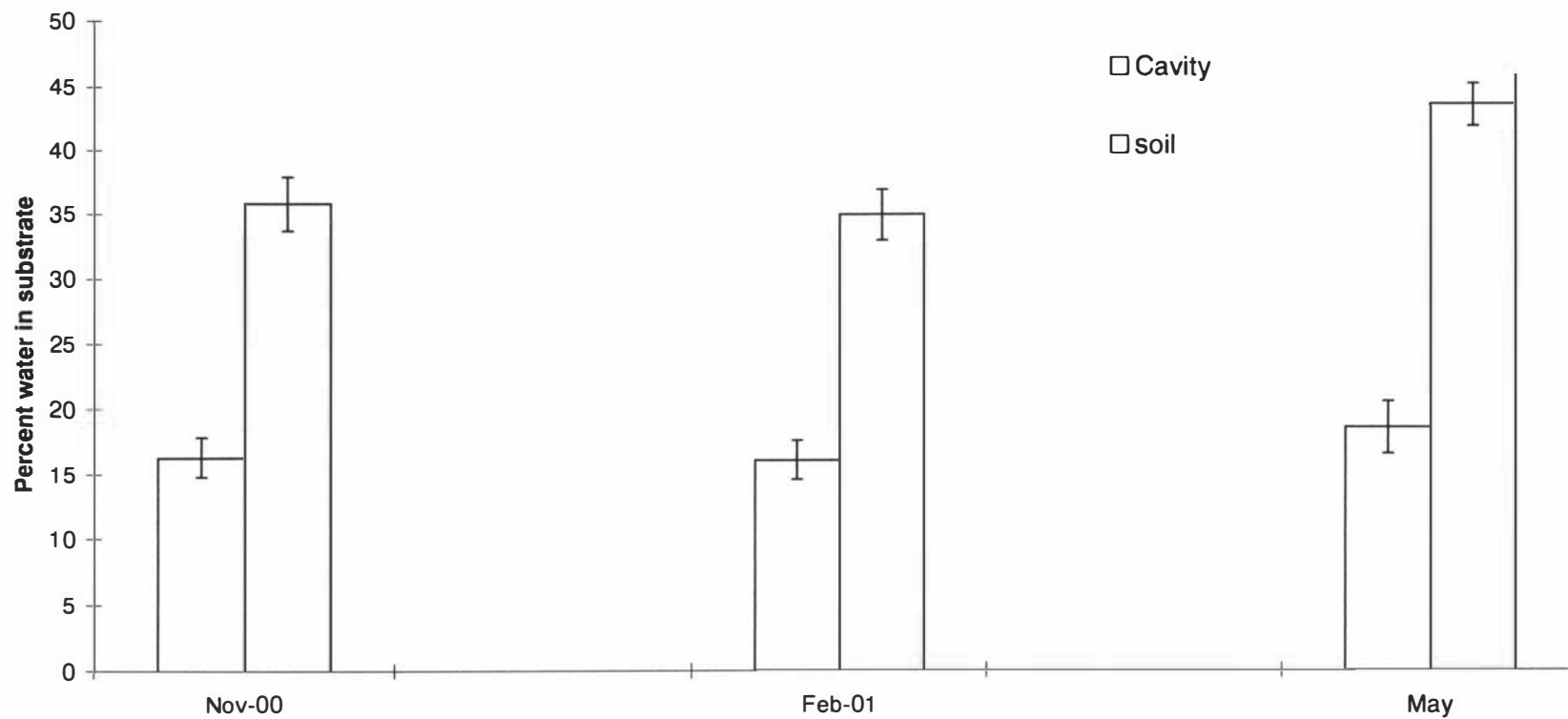
Figure 5.5. Monthly variations in percent water per gram of substrate for inside Puriri cavity-nests *vs* outside surface soils on Little Barrier Island. Bars indicate standard error.

Figure 5.6. *Aspergillus fumigatus* airborne spore densities collected from five nest boxes on Mokoia Island. Bars indicate standard error.

7) *Potential nesting material on Mokoia and Little Barrier Island*

Potential nesting material collected on Mokoia during October and November 1999 had a mean *A. fumigatus* density of 510,000 ± 192,000 CFUg^{-1}. Therefore, *A. fumigatus* densities recorded from potential nesting material were about three fold higher than actual nesting material, and about 1.3 fold higher than *A. fumigatus* densities recorded from surface soils at the nesting sites during the same period.

Potential nesting material collected on LBI during November and December 2000 had a mean *A. fumigatus* density of 29,000 ± 7000 CFUg^{-1}. These results indicate that *A. fumigatus* densities recorded from potential nesting material are abo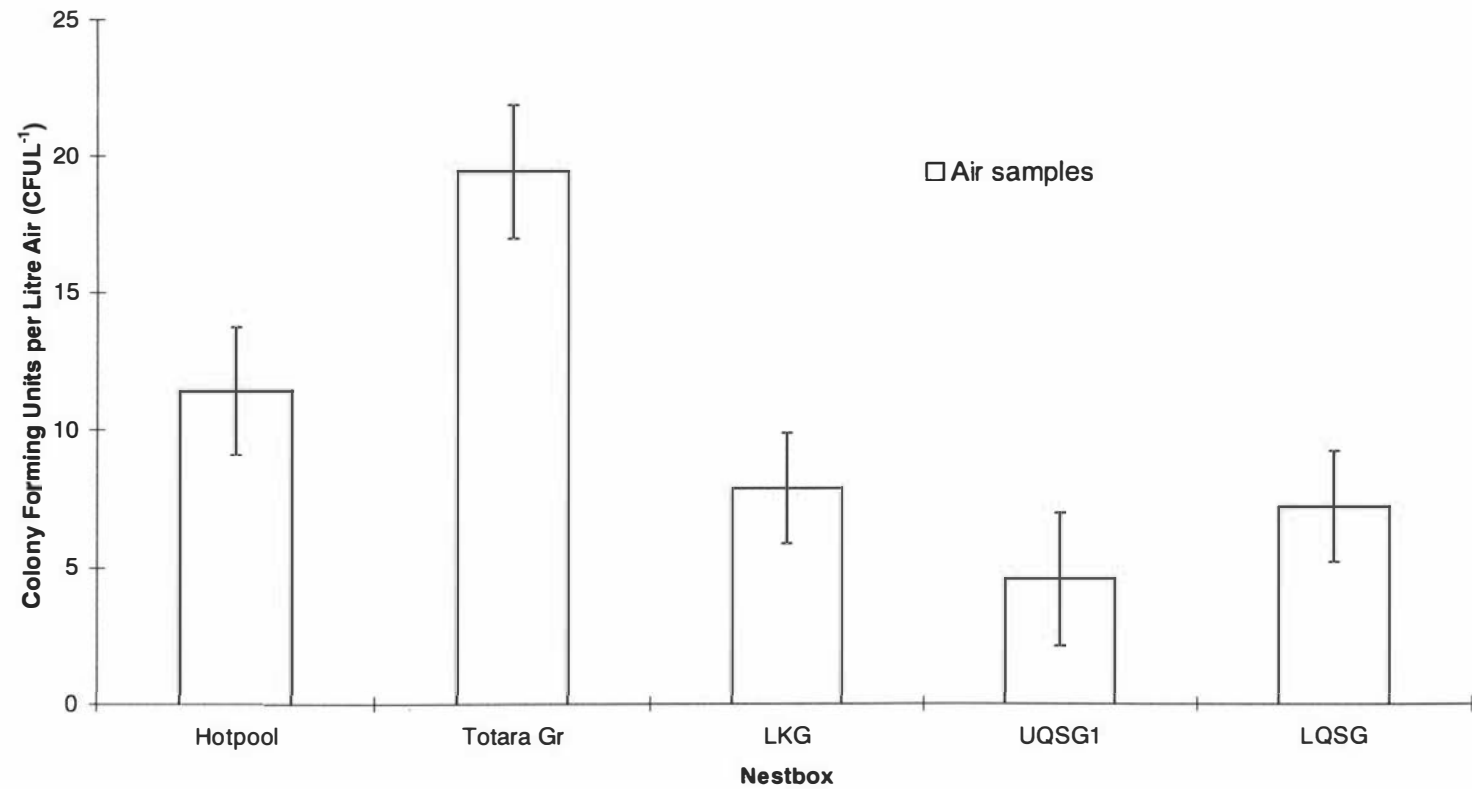ut 2.9 fold lower than actual nesting material, and about 1.5 fold higher than *A. fumigatus* densities recorded from surface soils at the nesting sites during the same period.

8) *Airborne* Aspergillus fumigatus *densities on Mokoia and Little Barrier islands*

While substrate samples on Mokoia indicate no increased exposure to *A. fumigatus* by hihi while on the nest, air samples collected at the end of the nesting period are in contrast to this. Airborne spore samples collected from inside hihi nest boxes on Mokoia ranged from 25 to 1.7 CFUL^{-1}, with an overall mean density of 9.32 ± 1.4 CFUL^{-1} (Figure 5.6) External air samples collected during October/November 2000 ranged from zero to 0.73 CFUL^{-1}, with an overall density of 0.33 ± 0.03 CFUL^{-1}. Therefore, on average airborne spores densities inside hihi nest boxes on Mokoia were about 13 times higher compared to airborne spore densities recorded outside the nest boxes.

No air samples were collected from air inside cavity nests on LBI. However, air samples were collected from outside each cavity-nest. On Little Barrier Island air samples collected outside tree cavity-nests ranged from 0 to 0.9 CFUL^{-1}, with an overall mean density of 0.1 ± 0.02 CFUL^{-1}. Airborne spore densities were about four times higher on Mokoia compared to LBI (see Chapter 3).

Discussion

Nest box nesting materials on Mokoia Island had *A. fumigatus* densities of around 200,000 ± 27,000 CFUg^{-1}. This demonstrates that hihi are exposed to *A. fumigatus* when

using nest boxes on Mokoia. However, this is about twice as low as the levels recorded in the surface soils outside the nest boxes during the same period on Mokoia, i.e., 401,000 ± 108,000 CFUg^{-1}. Additionally, chitinous materials in the nest box, such as discarded cicada remains had higher *A. fumigatus* densities associated with them than the nesting substrates derived from plant materials (i.e., 510,000 ± 46,000 CFUg^{-1}). In fact, these chitinous materials in the nest box returned mean *A. fumigatus* densities above estimates recorded in the surface soils outside the nest boxes. Therefore, it is possible that levels of chitinous materials in the nesting environment could further increase the overall levels of *A. fumigatus* contamination. This may partly explain why hihi on LBI build a fresh nest at a different location for the second brood.

Davis et al. (1994) studied the removal of old nest material by several cavity nesting bird species before constructing their second nest. In this study they documented the presence of Salmonella, a known microbial pathogen, in old nests. They hypothesised that one potential consequence of the removal of old nest material prior to rebuilding the second nest is the reduction of populations of potentially harmful micro-pathogens. However, avoiding old nesting sites is not an issue for Mokoia hihi because old nest boxes are removed at the conclusion of each nesting attempt and replaced with a clean nest box.

Changing nest boxes at the conclusion of each nesting attempt is one way of reducing mite and microbial pathogen loads in nest boxes. However, the provision of clean nest boxes may result in early colonisation by *A. fumigatus*. This is because *A. fumigatus* is a pioneer saprophyte with high reproductive and dissemination rates, and these traits enable the fungus to colonise 'virgin' substrates very quickly. It may be possible to control *A. fumigatus* levels within a nest box by establishing a non-pathological microbial community within the nest box prior to placement on Mokoia. This may involve taking soil from LBI, which is known to be low in *A. fumigatus*, and inoculating Mokoia nest boxes with quantities of LBI soil. By controlling the microbial community within the nest box we may be able to prevent *A. fumigatus* from getting an early foothold within the nesting material.

Air samples gave the strongest evidence that nest boxes on Mokoia are exposing hihi to elevated levels of *A. fumigatus*. Aspergillosis is a respiratory disease caused when a bird

breathes in sufficient numbers of spores to initiate an infection. These infections are usually acute in small animals, and usually result in death. Aspergillosis is presently considered to be incurable in wild bird populations. Therefore, preventative measures are important, and this requires information on where the most likely sources of *A. fumigatus* are. It is possible hihi chicks are breathing in spores while confined to the nest box and later developing an infection after fledging during hard times. The high humidity and constant substrate agitation that occurs inside active nest boxes may promote high airborne spore densities, and this may predispose breeding females and chicks to aspergillosis.

Since 1994 nest box management has became an integral part of hihi recovery on islands with young regenerating forests. Mokoia Island is such an island, and has few naturally available tree cavities. It is likely, that the provision of nest boxes on Mokoia is allowing hihi to reside in an otherwise unsuitable habitat. On Kapiti Island, hihi do not use Mokoia-type habitat, possibly because these areas lack natural tree cavities (Castro 1995). As mentioned, habitat use does not necessarily equate with habitat requirements (Grey and Craig 1991). The fact that hihi do not use Mokoia-type habitat on Kapiti does not necessarily imply that it is less suitable. Tieke, or saddlebacks, are another cavity nester thought to specialise in old growth forests. However, they have done extremely well on Mokoia and Tiri with nest boxes provided (Craig 1994; Armstrong and Craig 1995). However, as mentioned saddlebacks on Mokoia tend to nest away from the gullies, while hihi seem to prefer to nest in gully areas. Gully areas are cool damp areas during the breeding season, and these locations have higher *A. fumigatus* densities associated with them than ridge and summit areas. Therefore, providing nest boxes for hihi outside gullies may reduce levels of *A. fumigatus* within active nest boxes. However, results indicate that *A. fumigatus* densities were similar for all hihi nest boxes, and placement of the nest box seems to relate little to levels of *A. fumigatus* in nesting materials. Therefore, it is likely that saddleback nest boxes would have similar levels of *A. fumigatus* contamination. Why then are hihi suffering such high rates of aspergillosis on Mokoia and not saddlebacks? This may suggest that hihi are unusually susceptible to *A. fumigatus* infections, and/or that saddlebacks may have acquired higher immunity to *A. fumigatus* infections. By studying the immunological systems of hihi and saddlebacks on LBI and Mokoia, we may be able to manage hihi resistance to aspergillosis.

The theory of natural selection suggests that birds should prefer cavity nesting sites if use of these sites increases their breeding success. Nice (1957) and Lack (1968) demonstrated that the survival of nestlings is significantly greater in species that use cavity nests compared with species that use open nests. Increased nest attentiveness (i.e., proportion of time spent on the nest) should be required when local microclimate conditions are harsh and thereby require greater incubation feeding, i.e., the microclimate hypothesis. Because of energy limitations, variable microclimate nesting conditions may constrain nest attentiveness. The microclimate is more variable in open nesting birds compared to cavity nesting birds. The microclimate hypothesis should, therefore, predict that open nesting birds be more inclined towards higher rates of nest attentiveness compared to cavity nesting birds. However, time spent on the nest is most often greater among cavity nesting than among coexisting open nesting birds (Martin and Ghalambor 1999).

Castro *et al.* (In press) found that providing supplement food outside hihi nest boxes causes females to spend less time on the nest and less time foraging from natural food sources on Mokoia. If the natural food supply and nest boxes are a major sources of aspergillosis to hihi, then less time on the nest and reducing their dependence on natural food supplies may lower birds exposure to *A. fumigatus*. Therefore, supplement feeding may provide breeding birds with a healthier food supply, and allow birds more time to spend on disease prevention activities such as personal hygiene and sunning activities. It is possible that nesting behaviour among Mokoia hihi is influenced by the high levels of *A. fumigatus* on the island and within the nesting environment. Hihi on Mokoia may be abandoning nest boxes for similar reasons hihi on LBI avoid using old nesting material. Hihi might be sensitive to conditions that lead to high densities of disease-causing organisms, and may exhibit behaviours that allow them to avoid unnecessary contact with *A. fumigatus*.

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Chapter 6

General discussion

People judge the importance of disease outbreaks in different ways. One important factor that influences attitudes and responses to disease outbreaks is the degree to which humans are responsible for the outbreak. Diseases are a natural feature of life and most people accept that we should be very cautious about interfering with natural processes even if, like naturally occurring outbreaks of disease in free-living animals, they have unpleasant effects. However, if it is found that a disease outbreak in wildlife is due to human activities then many people feel that it is right that we should take steps to treat the problem and prevent it happening again. These are conservation and welfare concerns because the survival of human ecosystems are directly linked to the survival of 'natural' ecosystems. This study suggests that there is a link between the modifications of old growth habitats and the prevalence of avian disease causing organisms such as *Aspergillus fumigatus*. I suggest that this study illustrates that biodiversity, community structure and function in mature forests play an important role in keeping parasitic diseases and weed-like pest species in check. The irony is that this 'old-fashioned' single-species investigation provides direct evidence that supports the contemporary "ecosystem approach" in Conservation Biology, i.e., the paradigm that if endangered species like hihi are to be saved, then we must protect them against negative human impacts on ecological processes in mature forests.

Since Darwin, prominent biologists have hypothesised about the relationships between biodiversity and ecosystem functioning. Global concerns about declining biodiversity and questions regarding the degradation of ecosystem processes have stimulated a multitude of observational, theoretical, and experimental ecosystem studies over the past 25 years (Meffe and Carroll 1994; Debinski and Holt 2000). Most contemporary ecologists advocate an "ecosystem approach" to conserving endangered species; they point out that endangered species are almost always highly visible indicators of ecosystem health (i.e., Chase *et al.* 2000). However, the ecosystem approach to date has not been able identify with any clarity which ecological processes constitute ecosystem

health. The contemporary paradigm recognises that ecological systems are generally not in dynamic equilibrium, at least not indefinitely, and have no stable point. Identifying ecological processes is further confounded because the regulation of community structure and function is often not internally generated. External processes in the form of natural and unnatural disturbance events such as deforestation, selective or 'sustainable' logging, fires, floods, droughts, storms, earth movements, and outbreaks of disease or parasites frequently override internally generated ecological processes (Wilson and Peter 1988; Spalding and Forrester 1993). An important research goal for the ecosystem approach is to understand how the interactions between nonequilibrial processes and functional groups of species determines biodiversity and community structure in modified habitats. This study partly contributes to this goal by providing information on several ecological processes that can potentially contribute to differing disease levels recorded between different forest habitats. Some individual species such as hihi may be indicators of ecosystem health if an association exists between their presence and another component of biodiversity (Wilson 2000). This study suggests that transitory disturbance events generated during habitat modification favours the establishment of one of the world's most prolific avian disease causing organisms. If hihi are vanishing primarily due to common opportunistic diseases, then the plight of the hihi may indicate wider wildlife health problems facing other native bird species in New Zealand. Ecological indicators for long-term monitoring programs are needed to detect and assess changing environmental conditions (Spalding and Forrester 1993). This is because natural ecosystems are dynamic systems with fluxes of species, materials, and energy, and the prevalence of wildlife disease needs to be understood in the context of these changing environmental conditions. My results suggest that translocating hihi populations to modified or forest edge habitats creates an unhealthy situation similar to treating immuno-compromised aids patients in contaminated hospitals. Upsurges of other diseases such as malaria, cryptosporidiosis, giardiasis, trypanosomiasis, schistosomiasis, filariasis, and onchocerciasis have also been linked to habitat modification (i.e., Coluzzi *et al.* 1979; Coluzzi *et al.* 1985; Patz *et al.* 2000).

Epidemiology in an ecologically threatened world

Emerging diseases are increasingly being reported as causes of death in free-living wild animals (Daszak *et al.* 1999; Rogers and Randolph 2000). These diseases are of particular threat to free-living animals whose population, habitat, or range has been diminished or artificially manipulated to promote species survival, e.g., captive breeding, translocation, and release programs (Lyles and Dobson 1993; Anderson 1991; Viggers *et al.* 1993; Cunningham 1996). Greater numbers of epizootics and panzootics of wildlife are being reported in terrestrial (Daszak *et al.* 1999) and marine (Harvell *et al.* 1999) habitats and are probably underreported (Daszak *et al.* 1999; Cunningham 1996; Dobson and Hudson 1986; Scott 1988; Daszak and Cunningham 1998). Recent advances in theoretical and experimental host-parasite ecology has demonstrated that disease-causing agents can play a major role in the population biology of wild animals (Anderson and May 1986; Tompkins and Begon 1999).

Epidemiology of wildlife disease is the study of factors that facilitate or indirectly cause the development of disease in free-living animals (Anderson 1991). These facilitating factors are complex and usually interrelated. However, many wildlife diseases are characterised by long periods of dormancy, when little or no evidence of disease in the host population can be detected. At erratic intervals, sometimes separated by several decades, there is a sudden emergence of disease, often developing into an explosive epidemic. Studies on such epidemics frequently suggest that habitat and climate-related factors may have been primarily responsible for triggering these outbreaks (McMichael 1993; Wilson 2000). At present the timing of many wildlife disease outbreaks appear notoriously unpredictable. This creates a wildlife management problem because veterinary authorities are usually unaware of the existence of an epidemic until many weeks after its commencement and, therefore, unable to implement countermeasures until after many infections have already been contracted within the population. However, there are no short-term countermeasures for non-contagious infectious diseases such as aspergillosis in wildlife (Anderson 1991). If there is a link between habitat modification and the prevalence of this, and other wildlife diseases, how much longer can we continue implementing short-term single-species solutions to essentially long-term environmental problems? This further suggests that the epidemiologist's task must include participation in the development of ecosystem based strategies for preventive action.

Professor Aldo Leopold, one of the founding fathers of the discipline we now know as Conservation Biology, wrote 73 years ago "the role of disease in wildlife conservation has probably been underestimated". With a talent bordering on clairvoyance, many of his ideas remain current today. Today, wildlife managers typically include parasites and diseases on their lists of causes of mortality for many wildlife species. However, because there is usually little or no information on the natural occurrence of these disease-causing agents in the environment, they are often listed as a catchall afterthought without any specifics. This has created a situation whereby wildlife managers working with animals affected by parasites and diseases, such as the current situation with hihi on Mokoia Island, rely almost exclusively on the advice and assistance of trained parasitologists and wildlife veterinarians.

This study was initiated with the discovery that cicadas on Mokoia Island (Lake Rotorua) are contaminated with the fungus *Aspergillus fumigatus*, a common aspergillosis-causing pathogen in birds (Chapter Two). Mokoia hihi (or stitchbird, *Notiomystis cincta*) are known to be susceptible to aspergillosis and regularly feed their young on cicadas. Linking the disease-causing agent to the hihi food supply focused my attention towards identifying environmental factors related to the prevalence of *A. fumigatus*. Additional investigations established the presence of *A. fumigatus* contamination in floral nectar on Mokoia, another food item commonly consumed by hihi. Considering this and the general lack of information on the natural occurrence of *A. fumigatus* in any New Zealand forest, I decided to investigate whether *A. fumigatus* contamination can be correlated with habitat type, i.e., habitat contamination hypothesis.

Chapter Three provides evidence that *A. fumigatus* densities in surface soils may be positively correlated with habitat disturbance, in this case leaf litter removal. This may suggest that forest edge habitats subject to regular substrate agitation should have elevated levels of *A. fumigatus* compared to more stable inner forest habitats. This extension of the habitat contamination hypothesis is tested in Chapter Four and provides conformation that forest edge habitats and young growth forests have elevated levels of *A. fumigatus* compared to mature and inner forest locations. This information is important because it may suggest that hihi translocated to such habitats probably suffer

increased exposure to *A. fumigatus*, and therefore, more likely to develop *Aspergillus* type infections during lean periods.

Because many diseases such as aspergillosis are considered incurable in free-living bird populations, disease prevention information is critical to managing this disease. Chapter Five examines the hihi nesting environment on Mokoia and Little Barrier Island to determine whether nesting hihi and their young are subject to increased exposure to *A. fumigatus*, and whether habitat type and nest box placement has an influence on *A. fumigatus* contamination of nesting materials. Results from this chapter confirmed the presence of *A. fumigatus* contamination in used nesting materials from Mokoia and Little Barrier Islands. However, I found no evidence that nest box placement affects levels of *A. fumigatus* in nesting materials on Mokoia Island. At present there is no information available to determine whether levels of contamination reported in this thesis are sufficient to lower the health of hihi on Mokoia. A greater understanding of the natural occurrence of *Aspergillus fumigatus* and effects on birds immunocompetence is fundamental to developing effective aspergillosis prevention and control strategies to increase the success of future hihi translocations to modified environments.

Global implications

The growth and survival of many disease-causing organisms are usually linked to rather rigid host, environmental, and climatic tolerances (Wolfe *et al*. 2000). With habitat modification and climate change the prevalence and geographic distribution of many host-parasite systems are likely to change (Wilson 2000). For free-living species surviving on the very limit of their range, increased exposure to disease-causing agents could lead to an increase in disease related mortalities. For example, the parasitic disease avian malaria is a major mortality factor effecting native bird species on the Hawaiian Islands (Van Riper *et al*. 1986; Lindsay and Birley 1996; Redford and Richter 1999; Rogers and Randolph 2000). Birds become exposed to the protozoan parasites through the bites of mosquitoes, which can act as intermediate hosts for several malaria-causing protozoa (Van Riper *et al*. 1986). Both the mosquito and the parasite were accidentally introduced to the islands from Mexico about 150 years ago in tainted water barrels transported aboard visiting ships. Today, many of the remaining native Hawaiian bird species are confined to the high altitude forests scattered around the islands, where the low temperatures keep the mosquitoes at bay (Rogers and Randolph 2000). With

global warming it is likely the mosquitoes range will expand, and more Hawaiian native bird species will succumb to avian malaria (Rogers and Randolph 2000; Wilson 2000). With global warming and further habitat modification the prevalence of many opportunistic disease-causing agents such as *A. fumigatus,* may increase also (Spalding and Forrester 1993; Wilson 2000; Wolfe *et al.* 2000). With global warming, *A. fumigatus* development may begin earlier in the year and continue over an extended period of time. This may cause seasonally restricted windows for spore proliferation and disease transmission to expand, resulting in amplification of *A. fumigatus* populations in the environment, and in hosts. Ultimately, habitat change and global warming could result in the emergence of parasite-induced `opportunist` diseases. This situation may require us to take a second look at many parasite/host systems, and we may need to clarify with greater precision what we mean when we "pigeon-hole" parasites such as *A. fumigatus*, as 'opportunistic' disease-causing organisms.

Exposure is an important risk factor influencing individual host resistance to diseases like aspergillosis. This is because the infectious propagules produced by this parasite contain immunosuppressing mycotoxins, which can reduce an individuals ability to resist disease. This in turn could predispose some wildlife populations to poor health and diseases they historically had little trouble resisting at lower levels of exposure. At present, we are inadequately prepared to monitor and recognise the effects of habitat and climate change on host-parasite systems (Wilson 2000). Small scale empirical studies are not answering the most important questions regarding changes in the soil mycoflora following habitat change - is there currently a mass-extinction crisis going on in the soil? What is the functional significance of the immense microbial biodiversity in forest soils? Do changes in soil microbial diversity affect ecosystem functioning? Is habitat modification and resulting micro-climate changes causing a shift in the microbial diversity of forest soils?

According to Aldo Leopold our greatest challenge is to build on a new land ethic. "Most people think of land and preservation of land from a recreational standpoint, rather than a biological health standpoint. Can we move beyond symbolism and move beyond the concept of land for recreation to truly understanding the connection between the land and the integrity of the air we breathe and the water that we drink? We need to protect land so we can protect ourselves." Policy makers in New Zealand have begun to toy

with restoration ecology to suggest that we can justify destroying one natural area with the promise of simply re-building as we go. This study demonstrates that this notion threatens natural ecological processes and encourages the establishment of diseases that threaten our native bird species.

In 2001 little has changed for hihi, a single self-sustained population of the species still remain on Little Barrier Island. Hihi on other islands are either declining or holding their own with intensive management, i.e., Tiritiri Matangi Island. The hihi population on Tiritiri Matangi Island offer at least some hope, but even this relative success is scant protection for hihi if the species goes into decline on Little Barrier Island. With the acknowledgment that fungal disease is a major mortality factor affecting captive and free-living hihi (i.e., Alley *et al.* 1999), the future seems even more perilous for the species. Without serious intervention it is likely that hihi are only one very common disease away from complete extinction. It is not possible to overstate the importance to hihi of protecting the *status quo* on Little Barrier Island. Any further human impacts on the ecosystem that is Little Barrier Island should be viewed with great caution, because these acts threaten natural ecological processes and encourage the very diseases hihi seem most susceptible to.

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