Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

Syzygium jambos (L). Alston (Myrtaceae) control, conservation and restoration of the threatened native flora of Pitcairn Island, South Central Pacific.

Noeleen Smyth

Thesis submitted in fulfilment for the Degree of Doctor of Philosophy

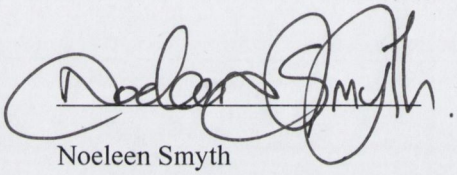
to

University of Dublin, Trinity College.

DECLARATION

This thesis has not been submitted for a degree to this or any other university and, with acknowledged exception, is entirely my own work.

I agree that this thesis may be lent or copied in accordance with Trinity College Dublin regulations.



Noeleen Smyth

ACKNOWLEDGEMENTS

There are so many people to thank for all their help and support over the years that it would take a second thesis to write down all the names. The most important people to thank are the Pitcairn Islanders, all of whom played a part in the "Roseapple team" and without their hard work and dedication on "Roseapple project" none of this would be possible.

A special note of thanks to Carol and Jay Warren the Project Manager and Conservation Officer who always went above and beyond the call of duty on a daily basis, and provided me with so much more than room and board during my stays on the Island; I am lucky to call them my second family. Thanks to all the staff of the Pitcairn Island office, Jenny, Shirley and Rosemary who took care of the project orders in New Zealand and helped with the logistics of getting to and from the Island. A special word of thanks to the Pitcairn Island Commissioner, Mr. Leslie Jaques OBE, whose unfailing support for Pitcairn means it's in safe hands. Thanks to the crews of "Braveheart" and the "Southern Salvor" who got me safely to and from the island on many occasions, through the roughest seas and craziest parties. Thanks also to the Ministry of Defence police on island especially Rick and Julie and the schoolteacher Mary and her husband Chuck.

Thanks to the Irish Research Council for Science, Engineering and Technology who provided funding through the EMBARK Initiative for the duration of my studies, even when I had to change project initially and get an extension. Thanks to the Foreign and Commonwealth Office and especially Mr. Shawn Earl who took care of the finances for the Roseapple project which were obtained from the Overseas Territories Environment Fund.

Thanks to the Botany Department and all the students; it is a warm and wonderful place to work, full of amazing and brilliant people. Dr. Trevor Hodkinson, Dr. Rhian Smith, Dr. Maeve Harbourne, Wood, Yanis and Lex were of great assistance in the molecular laboratory and Dr. Jane Stout was on hand for advice. A special word of thanks to the gang past and present of Room 9, the best folk you could meet; Darach-who lights and guides the way, Sarah-who motivates and listens, Anke, Caroline, Karl, Caoimhe and Ruth. Thanks to all my friends old and new, from the National Botanic Gardens, NPWS, my neighbour Anne and teacher Conchobar.

Thanks to my family for putting up with me through thick and thin, especially my brother Martin who is my rock, and my Mam Jo, the bravest woman I know, and to whom this thesis is dedicated. I lost Colm along the way but without his initial support I could have never started this journey, thank you all so very much.

Thanks to Dr. Steve Waldren the "super" supervisor, what can I say it's been so fantastic, thank you for the wonderful opportunity and all the help, and finally thanks to Dr. Naomi Kingston who has always been on there to provide support, knowledge and advice on the project and so much more.

Go raibh míle mhaith agat go léir

Abbreviations

AFLP	Amplified Fragment length Polymorphism
ANOVA	Analysis of Variance
Bp	Base pair
Bs	Bootstrap
C	Carbon
CBD	Convention of Biological Diversity
CCA	Canonical Correspondence Analysis
CR	Critically endangered
DCA	Detrended correspondence Analysis
DNA	Deoxyribonucleic acid
IUCN	The International Union for the Conservation of Nature and Natural Resources
LOI	Loss on ignition
N	Nitrogen
NMS	Non-parametric multidimensional scaling
P	Phosphorous
pers.comm.	Personal communication
pers. obs.	Personal observation
PCA	Principal Components Analysis
PCR	Polymerase chain reaction
RAPD	Restriction Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
rpm	Revolutions per minute
SER	Society for Ecological Restoration
sp.	Species (singular)
spp.	Species (plural)
UPGMA	Unweighted Pair Group Method with Arithmetic mean

Summary

To fully fulfil the many aspirations of plant conservation, a quantitative, practical and economic approach to the problems in hand must be taken. Modern conservation biology involves recovering lost habitat and thwarting exotic invaders, alongside single species conservation which requires some investigation into population viability and molecular genetics. In order to fulfil these aims for conservation efforts on Pitcairn Island, a logical approach was taken for this small island ecosystem, which can be used as a model for larger and more diverse islands, and fragmented landscapes on continents, which can be considered as "islands in a sea of land".

The initial investigation involved gathering baseline data on the effects of the invasive species *Syzygium jambos* on the native forest communities of Pitcairn Island. These investigations were carried out over a period of three years with annual expeditions ranging from two to three months on Pitcairn Island. The diversity, vegetation, soil characteristics, and density of *Syzygium jambos* invaded forest was characterised by means of quadrats and soil samples. *Syzygium jambos* was found to adversely affect species diversity in forested communities on the island, with diversity in *Syzygium jambos* invaded forest, less than half that found in native forest communities. A different suite of species was also found in *Syzygium jambos* invaded forest. The soil pH within *Syzygium jambos* invaded woodland (pH 5.86) was significantly lower than pH values found in native woodland communities (pH 6.60). The organic matter content also differed between invaded and native woodland communities. Organic matter was higher (23.38%) in invaded *Syzygium jambos* woodland when compared to native woodland (22.32%). *Syzygium jambos* was also found to occur at high densities on Pitcairn Island (seedling density $47.7m^{-2}$, sapling density $6.1m^{-2}$, and adult density at $2.3m^{-2}$). The features described above of *Syzygium jambos* invaded forest on Pitcairn Island are significantly different to native forest communities on the island and these features in turn may be facilitating further *Syzygium jambos* invasion on Pitcairn Island with species diversity and soil chemistry negatively affected by its presence.

Developing suitable methods of control for *Syzygium jambos* was the next step in the process. Two different physical treatments: *cutting,* where trees were cut down and removed from the site or *frilling,* which involved removing the bark from around the base of the tree and leaving the tree standing in-situ and two different chemicals were also used, Roundup® or Tordon®, which were applied as a high volume spray to cut and frilled trunks: these treatments were applied over a two year period. The most suitable and most economic method for *Syzygium jambos* control on Pitcairn island was found to be frilling using the chemical Roundup® which gave high *Syzygium jambos* mortality rates (98.96% in 2005), had cheaper capital and running costs as well as being less labour intensive (66 person hours per $400m^{-2}$) and was considered less dangerous on steep slopes by personnel.

A recovery programme to restore elements of the native forest community after *Syzygium jambos* control was the next challenge. Experiments into the composition of the soil seed bank in

invaded forest was found to deficient in native species and indicated that weedy species were the likely replacement vegetation to *Syzygium jambos* in treated sites. Direct seeding of the native trees, *Homalium taypau*, *Meterosideros collina* and *Hibiscus tiliaceus* in treated sites had very limited success. A plant propagation and growing facility was built on the island in 2003, in order to grow replacement species for the treated sites. Twenty eight different species were grown, which were representative of species found in native woodland communities as described by Kingston and Waldren (2005) and some rare endemic and native trees and shrubs. Of the 1,400 plants planted in sites during 2003 and 2004, 833 survived. The mean percentage plant survival in treated sites was 59.5%. Highest survival rates were found for sites where *Syzygium jambos* was frilled and the site was weeded (80%). Plant survival was lowest in sites in which *Syzygium jambos* was cut and the site subsequently weeded (42%) though this treatment exhibited the highest plant growth rate (70.8cm in 10 months). The causes of plant mortality were drought (57%), goat browsing (37%), falling debris (3%) from dying frilled *Syzygium jambos,* and washout (3%).

The final challenge was to conserve some of the remaining populations of threatened rare and endemic plant species on the island. An assessment of the genetic diversity and threats, both demographic and environmental, were made where appropriate for five critically endangered species; *Abutilon pitcairnense, Myrsine* aff. *niauensis, Haloragis* sp., *Coprosma benefica* and *Lastreopsis* c.f. *pacifica. Abutilon pitcairnense* was successfully propagated by cuttings and seed. The seedlings obtained from the original genotype were characterised by a molecular fingerprinting technique (AFLP), five distinct genotypes of this species are now conserved in cultivation and genetic diversity remains high (H_T0.346) in comparison to other rare species. The sole refound individual of *Myrsine* aff. *niauensis* was also successfully propagated by cuttings. Molecular sequencing of the *trnl-f* region of the chloroplast genome revealed that the plant on Pitcairn was genetically most similar, to the Henderson Island species, *Myrsine hosakae*. Populations of *Lastreopsis* c.f *pacifica* and *Haloragis sp.* were monitored over the three years and the populations on Pitcairn were found to be stable. The population of *Coprosma benefica,* which consisted of 12 dioecious individuals in 1997 (6 female, 1 male and 4 unknown), had decreased to 11 individuals by 2003 (8 female, 1 male and 2 showing both male and female expression). This knowledge combined provides for recovering the lost native forest habitat on Pitcairn Island and thwarting the exotic invasive species *Syzygium jambos*, alongside single rare and threatened species conservation and recovery efforts. The holistic approach taken here to both habitat and species recovery was both demanded and required to fulfil the aspirations set out by the term "conservation biology".

Table of Contents

Chapter 1

Introduction

1.1 Introduction to Oceanic Island ecosystems

Island floras, particularly oceanic island floras, are often low in biological diversity due in part to their isolation and small size in comparison to continental areas. Despite this, they are of great scientific interest, mainly due to the occurrence of many unique elements in their biota (Cronk 1997). One of these elements, the high degree of endemicity, elicits great interest from the scientific community and was one of the four features of island floras first highlighted by Hooker in a lecture to the British Association in 1866. The other elements he highlighted as features of island floras in his lecture were impoverishment, dispersal and disharmony (Berry 1992).

Island endemics can be one of two types; *paleoendemics*, which are remnants of basal lineages that have become extinct elsewhere and have found refuge on islands, or *neoendemics*, the recent products of speciation events from lineages which still occur elsewhere (Whittaker & Fernández-Palacios 2007). Island endemics offer scientific insights into the processes of dispersal, colonisation, evolution and, all too often, extinction (Quammen 1997). The low diversity on islands also allows for studies of ecosystem functioning, with the simpler island ecosystems offering valuable insights into more complex systems elsewhere; these replicated and simplified systems allow scientists to isolate factors and processes and explore their effects (Whittaker & Fernández-Palacios 2007). Scientific studies on islands have been of immense importance in developing many modern biological and evolutionary theories. The most famous example, the theory of evolution by natural selection, put forward by both Darwin and Wallace who arrived at similar conclusions after extensive periods of research on islands (Waldren 2002).

Islands were first classified into three types by Wallace in his book entitled *Island Life* (3rd Edition 1895) according to their geological origin and biological properties, long before knowledge of plate tectonic theory and the influences of glaciations became accepted. Despite that, his classification theory is still of fundamental importance (Whittaker & Fernández-Palacios 2007).

1. **Recent continental islands**: are emerged fragments of continental shelf separated from the continents by narrow, relatively shallow waters. This recent separation as a consequence of postglacial sea level rises, isolated species on islands from their mainland relatives, the speciation process is limited. The number of endemics is generally low but can be variable depending on latitude, often low numbers of endemics in high latitudes and a high number

of endemics in low latitudes. Examples of recent continental islands include Britain, Ireland, Trinidad, the Falklands, Tasmania, Borneo and New Guinea.

2. **Continental fragments**: or ancient continental islands, which were once part of continents tens of millions of years ago, and which are separated now as a result of tectonic drift from the mainland. The waters between them and the continents are wide and deep. The long period in isolation has allowed the persistence of some ancient lineages and the development of new species *in situ* by vicariance. Endemism on these types of islands tends to be very high. Examples of continental fragment islands include Sicily, Crete, Cyperus, Jamaica, Madagascar, Seychelles, New Zealand and New Caladonia.
3. **Oceanic islands**: originate from submarine volcanic activity, are built over the oceanic plate and are of volcanic or coralline formation, they are remote and have never been attached to continents. They are populated by species that have dispersed to them from elsewhere and have subsequently been enriched by founder effect speciation and subsequent radiations. Endemism on these islands tends to be high. Examples include Iceland, Canaries, St. Helena, Hawaii, Galápagos, Society Islands and Pitcairn.

Oceanic islands throughout Polynesia have been further classified by the diversity of their physical environments by Fosberg (1991) (Figure 1.0):

Class 1: Young active or recently active volcanoes with surfaces of hard lava flows, or of ash, cinders or pumice. The rock in eastern Polynesian volcanoes is basaltic mix of iron and magnesium silicates and neutral in nature. In western Polynesia the rock is andesitic with aluminium silicates and slightly more acidic. These islands tend to be high and conical in west Polynesia and broad and sloping in east Polynesia. Soil is scanty and streams occupy ravines on lower slopes. An example of a young, recently active island is Pitcairn Island.

Class 2: Older dissected volcanic islands, which are deeply weathered and much dissected, slopes are steep and the upper ridges are sharp. Soils are deep on gentler slopes and leached and acidic on wet windward sides. Permanent streams are frequent and waterfalls are common. They often have a flat coastal strip and are surrounded by fringing reefs. An example of older volcanic islands, include many of the Society Islands for example, Tahiti.

Class 3: Almost-atolls, the islands are low and much eroded and worn in extreme cases, for example Clipperton Island which is a mere rock, steep and rugged.

Class 4: Coral atolls which are ring shaped reefs with often many islets of coral limestone. Coral atolls are found frequently throughout Polynesia and examples are frequent and include Oeno and Ducie islands, in the Pitcairn group (Figure 1.1).

Class 5: Elevated atolls which are platforms of coral limestone raised 30-100m above sea level, some soil exists on the upper platform surface but most areas are dissected, pinnacles

of sharp limestone. Henderson Island, one of the Pitcairn group of islands and a World Heritage Site (Figure 1.1), is thought to represent an example in its more or less its original state, as many of the others have been mined as they contain large deposits of calcium phosphate.

Class 6: Mixed Volcanic and Limestone islands called *Makatea* by the Polynesians. These low volcanic islands have terrace-like limestone masses which rest on volcanic slopes, which can be found well above sea level or extending up from sea-level. The volcanic rock is deeply weathered though there is little soil on the limestone. Many examples of these are found in the Austral and Cook archipelagos (Figure 1.0).

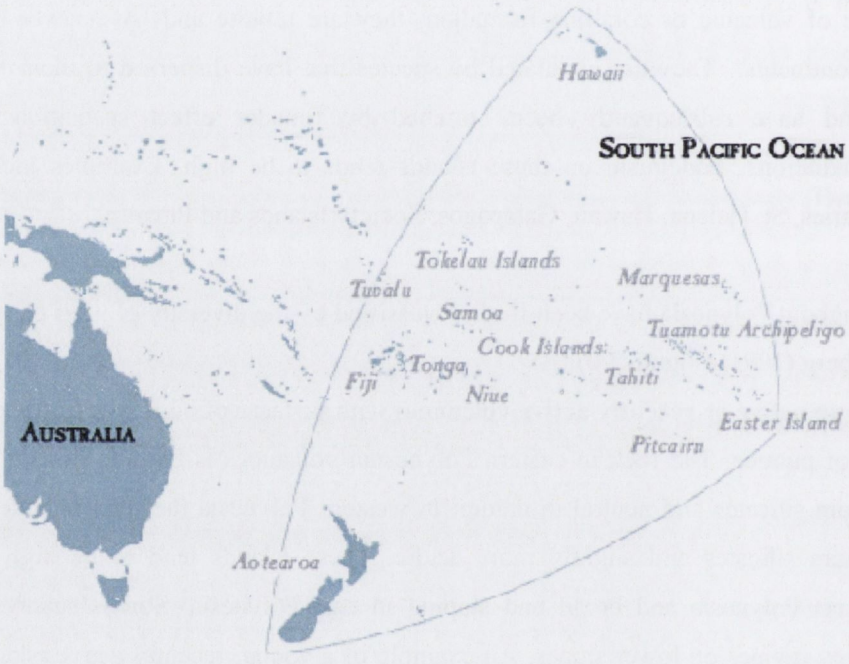


Figure 1.0: Map of Polynesian Islands

1.1.1 The ecology and evolution of oceanic Island ecosystems

In 1967 MacArthur and Wilson published the equilibrium model of island biogeography (EMIB) who proposed that the species number on an island was the dynamic product of opposing rates of immigration and extinction as inverse functions of isolation and area. They also proposed that the greater the area of an island, the greater the number of species (Whittaker & Fernández-Palacios 2007). However, this theory in many instances does not hold true, with many exceptions to the rule and it is difficult to apply. Distinguishing between a species that has immigrated and one that is in transit or in insufficient numbers to found a population is very difficult (Williamson 1988; Whittaker & Fernández-Palacios 2007).

Oceanic islands are a "blank canvas" and receive all their biota when they arise through long-distance dispersal (Paulay 1994), with the species composition on each the result of individual species' dispersal ability, the age of the emergent island, the remoteness of the island, the size of

the island, the available habitat niches and adaptive radiation (Quammen 1996; Whittaker & Fernández-Palacios 2007). On oceanic islands in the Pacific, biotic diversity is limited and often the populations are very small, the species range is also very limited and extinction is a frequent occurrence (Fosberg 1991).

The starting point, dispersal across a pre-existing barrier through long-distance dispersal, can take place via ocean drift or **hydrochory** – for example, the typical strand plants in Polynesia such as *Cocos nucifera* and *Pandanus* species disperse this way; air currents or **amenochory**-for many light spored or seeded species such as pteridophytes, bryophytes and small seeded angiosperms such as *Meterosideros* species in Polynesia; and animals or **zoochory**, which can be further subdivided into passive zoochory or **ectozoochory**- for species which have sticky or barbed fruits such as *Bidens* and *Pisonia* found across Polynesia, or active zoochory or **endozoochory**-species with berried fruits such as *Ficus* species and *Coprosma* species in Polynesia (Kingston *et al.* 2003).

Once established, island plant species which are hermaphroditic with self-compatible reproductive systems have better chances of colonization than dimorphic or obligate out-crossers (Bakker 1955). Data suggest that the floras of Hawaii (Carr *et al.* 1986), New Zealand (Webb & Kelly 1993) and the Galápagos (McMullen 1987) do have smaller proportions of self-incompatible taxa than nearby continents. Some species such as *Fragaria chiloensis* are dioecious in America but hermaphroditic in Hawaii and *Coprosma pumila* which is dioecious in New Zealand but monoecious in Macquarie (Ehrendorfer 1979). In many instances, island floras have high levels of dioecy or heterostyly; for example, 15% of the Hawaiian flora are strictly dioecious (Whittaker & Fernández-Palacios 2007), the reason why is intriguing. One reason perhaps is that out-crossing species will have high levels of genetic variability and thus be able to diversity into a range of available niches (Richards 1997). Genetic variability can be increased after establishment by mutation and re-sorting. An important form of re-sorting is genetic drift which is the chance alteration of allele frequencies from one generation to the next, which may be particularly important for small populations on islands. It is possible to demonstrate the occurrence of genetic drift on islands, for example, a study on a small population of house mice in the Orkneys revealed genetic drift (Berry 1992). An interesting study on endemic *Silene* in Hawaii also demonstrates that fact that populations on the older island of Maui are more polymorphic than those on the younger island of Hawaii, suggesting that genetic variation has been lost in the colonization of the younger island. This is an example of the founder effect (Westerbergh & Saura 1994). However, the contribution of founding effects and genetic drift to island speciation still remains a subject of debate (Barton 1989; Clarke & Grant 1996; Whittaker & Fernández-Palacios 2007).

One of the most interesting evolutionary forces which occur on islands is adaptive radiation, which is the evolutionary development of distinct species (or varieties) from a single ancestral form this evolution allows for niche specialisation. This can be the driving evolutionary force on oceanic islands as open habitats are found frequently, and isolation by erosional dissection

and lava flows are common, micro-species, subspecies and varieties often characterize island biotas (Fosberg 1991). There are numerous examples of species which have adaptively radiated on islands, such as Hawaiian honeycreepers and fruit flies, the most famous example being the Galápagos finches (Townsend *et al.* 2003).

1.1.2 Threats to Island Ecosystems

The single greatest threat to island biota and ecosystems are disturbances associated with human activities. The destructive effects of humans activities and associated introductions was first noted by Wallace in 1895, when he highlighted the destruction on St. Helena following European discovery, by goat trampling, forest clearance and the introduction of plant species. Humans are considered the main drivers of species range shifts and extinctions, which have become accelerated in more recent years (Bush 1996). Through human intervention, the bounds of species isolation have been changed, in many cases island species which have lasted for millions of years in isolation, and in the case of oceanic islands for their entire existence, are faced with new competitors (Whittaker & Fernández-Palacios 2007). Island plant species are under threat from invasive species and habitat destruction, it is estimated that one in three of all known threatened plant species are island endemics (Whittaker & Fernández-Palacios). Other dramatic and convincing illustrations of extinctions due to human activity come from islands worldwide (Diamond 1995). Primack and Ros (2002) also suggest that more than 99% of recent species extinctions can be attributed to human activities. Extinctions of vertebrate species over the last 400 years average 20-25 per century, a rate between 20-200 times the natural rate (excluding mass extinction events) and also a result of mans activities (Groombridge and Jenkins 2002).

While the extinction threats to island populations are perceived as relatively greater than those for continental regions, there is a lack of accurate, quantifiable information available on the status of island plant communities and their constituent populations. Accurate survey of remote islands is costly in terms of time, human effort and finance. In addition to remoteness, many 'high' islands of volcanic origin have difficult rugged terrain; so many populations may be inaccessible to surveyors. A further problem results from the frequent dissociation of the biodiversity hierarchy; all too often the focus of survey work is the distribution and extent of native vegetation communities, or population census of a particular threatened taxon (Waldren 2002).

The four features of island ecosystems- endemism, impoverishment, dispersal and disharmony (Berry 1992)- which make them of immense scientific interest also lend them vulnerable to disturbance. Species poverty on islands lends more vacant niche space to introduced species, species on islands tend to have a reduced competitive ability due to repeated "founder effects", island populations tend to be small and are more susceptible to change and susceptible to disease, and there is an exaggerated effect of ecological release for introduced species without their natural competitors and predators (Cronk & Fuller 1995; Cronk 1998; Simberloff 1998).

The two main types of extinction events operate on islands, these are *stochastic* and *deterministic*. *Stochastic* events are often inherent to the natural dynamics of many islands environments, for example:

- Natural disasters – volcanic eruptions, landslides, hurricanes and tsunamis
- Taxon cycle dynamics- demographic collapse and inbreeding depression
- Pleistocene climatic fluctuations (including sea-level rise)

Stochastic events can be further categorised into *environmental stochasticity*, *genetic stochasticity* and *demographic stochasticity*. Catastrophes, such as hurricanes, fires, droughts, volcanic eruptions, may have profound influences on the survival of populations, or even of species. Environmental stochasticity is likely to affect even large populations, the long term effects will depend in part on how rapidly the population can recover in relation to the frequency of stochastic events. Increased storm frequency, one result of global climate change, increases environmental stochasticity, thereby increasing the threats of hurricane damage to island vegetation (Waldren 2002).

Genetic stochasticity results from random changes in allele frequencies, due to founder events, genetic drift or inbreeding. The effects of genetic stochasticity on a species will depend on the breeding system, but may be expected to influence small populations; a frequently quoted figure is that effective population sizes of less than 500 individuals are likely to be prone to genetic stochasticity. The value of these figures to define minimum viable population sizes, often referred to as the '50-500 rule' (Shafer 1990), which are theoretically derived from data from populations of large vertebrates, and may not be of use or necessarily apply to island populations of other biota. This may be especially be true for island plant species, which may have already passed through genetic bottlenecks during colonisation. In addition, the generally more varied breeding biology of plants compared to most animals may mean that different and possibly lower minimum viable population sizes may be appropriate to plants (Waldren 2002).

Demographic stochasticity results from variation in the survival, fecundity and reproduction of different individuals within a population. The general rule of thumb is that only very small populations, those with an effective population size of less than 50 individuals (Shafter 1990), are likely to be prone to demographic stochasticity. In these considerations, the *effective* population size is the number of interbreeding individuals: which may in many instances be considerably less than the *total* population size (Wright 1931 & 1946). Again the relevance of this number to island plant populations can be called into question as many species on islands exist in extremely low numbers (Waldren 2002).

Deterministic extinctions are directly or indirectly related to human activities, for example:

- Habitat loss, degradation, or fragmentation
- Alien species introduction (including competitors, predators, hybridizing congeners, parasites, disease vectors or diseases)
- Direct predation (including hunting, fishing or specimen collection)

In global terms the loss of habitat is considered the greatest problem for biodiversity (Lawton & May 1995). The most renowned case of habitat degradation occurred on Easter Island on which unsustainable exploitation of natural resources caused the catastrophic extinction of many of the islands species and a human civilisation. Large trees were systematically felled in order to facilitate moving the giant statues for which the island is famed (Bahn & Flenley 1992).

Similar prehistoric examples abound from other Pacific islands where human populations disappeared due to overexploitation of natural resources, including animals, vegetation and soils (Diamond 2005). In more recent times lowland forests, and especially the dry lowland forests, of the central Polynesian islands have been similarly degraded or removed, being replaced by agriculture and savanna or plantations of non-native tree species (Mueller-Dombois & Fosberg 1998). The reduction in native forest on islands has in particular resulted in the loss of numerous island endemics (Steadman 1997 a &b).

However, it is alien invasive species that have caused the most destruction on islands. As humans have spread across islands, a remarkable array of either purposeful or inadvertent plant or animal and other taxa including microscopic species have followed. These anthropogenic species introductions are variously coined as exotic, alien or non-native species (Richardson *et al.* 2000; Whittaker & Fernández-Palacios 2007). The impacts of these introduced species have been described as "immense, insidious and usually irreversible" (IUCN 2000). Introduced species among other impacts can affect the balance of predators and browsers (Cronk 1989, Benton & Spenser 1995) and change pollination and dispersal networks on islands (Olesen *et al.* 2002). Introduced species are most typically those introduced as an ornamental or timber resource, examples of invasive trees on islands introduced for timber include *Syzygium jambos* on Pitcairn Island, and for ornamental value *Miconia calvescens* on Tahiti. *Miconia calvescens* dries out the soil in which it lives with resulting understory death of native tree ferns and other pteridophytes (Given 1993). Other reasons for species introduction include those introduced for ornamental value, for example, *Lantana camara*, which is considered responsible for the extinction of at least one endemic plant species from the Galápagos Islands (Mauchamp *et al.* 1998). Accidental introductions of alien species can also occur, for example grasses and other herbs introduced with crop seed or grain. Non-native invertebrate pests and plant diseases may also severely effect isolated island populations, which have not evolved in tandem with pests, diseases or predators. Disease may reach epidemic proportions, such as avian malaria carried by an introduced mosquito *Culex quinquefasciatus* which has been implemented along with habitat destruction, hunting,

competition, introduced birds and predators, as a major causal factor in the extinction of many of Hawaii's bird species (Van Riper *et al.* 1986).

However, deterministic threats rarely directly result in species extinction, though once the number of individuals has become reduced, stochastic threats play an increasing role in a species demise, which leads in many instances towards extinction, an "ecosystem transformer" species followed by a trophic cascade (O' Dowd *et al.* 2003).

1.2 Conservation of Island floras

Conservation biology is the science of maintaining the earth's biological diversity (Hunter 1995). Deterministic effects on islands, which are largely human induced, operate largely at the community level and may reduce population sizes considerably, but there is some scope for human intervention by recovering lost habitat and thwarting exotic invaders to redress the situation (Fiedler & Karieva 1998). Once population sizes are severely reduced, stochastic threats become increasingly significant; these threats operate mainly on populations of individual species or taxa, and there is no simple management option to regain control of the situation. Recovery plans for critically endangered species generally aim for a significant increase in both the population size and genetic diversity of the surviving population (Waldren 2002). However, the ideal we should strive for is protecting a representative array of ecosystems and habitats, which will protect biodiversity at both the species and genetic level to significant extent (Hunter 1995).

1.2.1. Habitat restoration and Island ecosystems

To reverse the trend of degradation and extinction, Hunter (1995) states that we must now think in terms of improving and restoring damaged ecosystems. Young (2000) predicted that restoration ecology will play an ever increasing role in biodiversity conservation and that the long term future of conservation biology is restoration ecology as most conservationists agree that habitat loss, climate change, invasive alien species, overexploitation and pollution are the greatest threats to biodiversity (Millennium Ecosystem Assessment 2005)

The practice of restoration ecology has been defined by the Society for Ecological Restoration (SER) as "the process of assisting the recovery of an ecosystem that has been degraded damaged or destroyed" (SER 2002). Restoration ecology has emerged as a more "optimistic" (Young 2000) approach to biodiversity conservation and was described as a "marriage of ecological theory and practical application" by Suding (2005). The major professional body *The Society for Ecological Restoration* (SER) was founded in 1988 and the first truly international meeting on Restoration Ecology and sustainable development was held in Zurich, Switzerland in 1996. All too often this "marriage" between the practice of restoration and the science of ecology is rocky, because restoration ecology is more like "battlefield medicine" where all too often action

needs to be taken with imperfect scientific knowledge (Van Andel & Aronson 2005), and practical knowledge often develops separately from hard scientific research (Packard & Mutel 2005).

The first recorded effort to recreate a natural community using ecological principles was conducted in 1935 by Aldo Leopold. He set out to restore American prairies which were vanishing due to agriculture and the demise of the bison (Jordan *et al.* 1987). In more recent times rehabilitation of damaged ecosystems, especially industrial sites, has been dominated by landscape architects and civil engineers who were reliant on agricultural practice rather than ecological theory (Clark 1997), and in many cases the aims are to restore *any* vegetation cover rather than the re-establishment of any pre-existing natural community.

Restoration in the strict sense is formally defined as returning a damaged ecosystem to its undamaged state. The goal is the creation of an ecosystem similar to a specified reference ecosystem that existed previously or exists at a particular nearby site (Bradshaw 1997). Rehabilitation, on the other hand, is defined as any improvement of degraded site, for most projects the goal is restoration but the actual achievement is rehabilitation (Bradshaw 1997), or restoration in the broadest sense. Selecting one ecosystem state fixed in time and space as a reference for restoration can be a "trap". A broader habitat type must be used to develop a framework that suits a particular site (Pickett & Parker 1994).

On most islands, the main phase of habitat clearance has passed and many are moving towards tourism, though with tourism a delicate balance has to occur if it is to be sustainable: large scale tourism developments are extremely detrimental (Hess 1990). The decline in agriculture on many islands offers the opportunity to expand through restoration areas of habitat adjacent to retained areas. Restoration or rehabilitation of island environments is not a new topic: J.R Forster (Grove 1995) in the 18th century proposed planting to improve the degraded landscape of Easter Island (Rapa Nui). Restoration of species should seek to integrate with local agricultural needs and landscape services for example, restoration work on indigenous tree fern thickets on St. Helena is working within the context of watershed security, and weed control of *Lantana camara* in the Mascarenes benefits both the conservationist and the local grazier (Maunder *et al.* 1997).

1.2.2. Species conservation and Island ecosystems

The biological definition of a species propounded by Mayr (1942) and which has gained much favour, defines species as "groups which are reproductively isolated from other such groups". This definition is relevant mainly in zoological terms because plant species are harder to define in terms of reproductive isolation and because they are likely to exhibit asexual reproduction, self fertilization and polyploidy (multiple sets of chromosomes). In conservation biology the fallback definition of a species "what a competent taxonomist says it is" (Stevens 1990) is often more relevant. The desire to seek and maintain all distinguishable taxa whether or not there is full agreement on the definition of a species, is more in tune with the aims of conservation biologists

(Hunter 1996). Species conservation is often seen as a "crisis discipline" with neither time nor adequate resources; it then becomes necessary to prioritise species based on a measure of their value, both taxonomic and genetic distinctiveness and the degree of threat (Hunter 1995). The goal of species conservation is to maintain the evolutionary potential of a species by maintaining natural levels of diversity. To effectively manage rare and threatened species, a plan must be drawn up to include information on the species ecology, breeding system, life-history and genetic diversity (Hamrick *et al.* 1991).

The use of molecular markers in species conservation has become increasingly popular as the genetic diversity within a species can help inform suitable conservation efforts, and is becoming widely used as techniques become automated and kits have been developed (Karp *et al.* 1996; Ouborg *et al.* 1999). Many types of molecular markers are available and widely used such as RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), minisatellite fingerprints and microsatellites or SSR (simple sequence repeats). Each marker differs in the type and amount of variability they express, in suitability for each particular question in relation to the species and population under investigation, and the ease of use and costs of the different techniques vary. The techniques and suitability are discussed in the following papers (Bruford *et al.* 1992; Avise 1994; Olmstead & Palmer 1994; Weissing *et al.* 1995; Jarne & Lagoda 1996; Ouborg *et al.* 1999; Bensch & Åkesson, 2005; Meudt & Clarke, 2007). Molecular markers can be classified as codominant (Microsatellites and most RFLP) where banding patterns of homozygotes can be distinguished from heterozygotes, or dominant (RAPD, AFLP) where banding patterns cannot distinguish between homozygotes and heterozygotes. Codominant markers allow for calculation of allele frequencies in populations, whereas dominant markers allow only estimation of genotype and not allele frequency. Minisatellite fingerprints are codominant but if a large number of bands are produced it is often difficult to calculate allele frequencies (Ouborg *et al.* 1999).

Any reduction in the population size of a species can dramatically reduce the genetic diversity within a species (Mauchamp *et al.* 1998). Maintaining rare and threatened species and the diversity within them in many instances requires active conservation measures. *Ex-Situ* conservation is the conservation of a species outside its natural range and *in-situ* conservation aims to conserve species in their natural habitat (Brown & Briggs 1991). The two methods can be considered complimentary. *In-situ* conservation however, is considered the ideal in that species are conserved in their natural habitat, but it is a complex task which requires links with habitat conservation effort (Given 1994). Where single threatened populations are vulnerable to extinction, the establishment of a second population in similar habitat can act as an insurance policy against stochastic threats which can cause the rapid extinction of small populations (Maunder 1992). The most famous case of an island species conservation programme comes from Easter Island (Rapa Nui). In the 1960's the last specimen of *Sophoro toromiro* (Fabaceae) was felled for firewood. A programme of genetic screening using RAPDs (Welsh & McClelland 1990; Williams *et al.* 1990)

confirmed that a number of *Sophoro toromiro* existed in *ex-situ* collections in Chile, Europe and Australia. A breeding programme using stock from all the *ex-situ* collections was used to enhance and maintain the genetic diversity of the species, and a reintroduction of the species back to Easter Island (Rapa Nui) was planned (Maunder *et al.* 2000) though no progress on this reintroduction has been reported in the literature since.

1.2.3 Integrated conservation and development on islands

Development options for small islands range from those that have no adverse environmental effects, for example, wildlife sanctuaries, to those with little impact or "subsistence affluence" economies, in which local needs are fulfilled though the sustainable use of local resources. The most extreme development options are those which bring about radical changes in the environment, for example, large scale tourism. The first option excludes human residents so is not really "development" in any true sense. The second, more ideal option can be hindered by growing populations and the aspirations of local peoples. The third option depicts what most of the world's islands have suffered, a "boom and bust" cycle of resource exhaustion and chronic emigration. Between these extremes lie development activities which "tread lightly" on the environment, such as fisheries, tourism, light industry and services (Beller *et al.* 1990).

Integrating species and habitat conservation have typically centred on two approaches, the establishment of national parks and other protected areas, and species recovery actions (Waldren 2002). There is a large literature on the best methods for reserve selection and design (Diamond 1975; Mittermeier 1998; Simberloff 1998): these debates are now considered banal (Fiedler and Karieva 1998) in the face of the obvious principle that a large reserve is better than a small reserve. More recent research has focused on the development of computer models which use an iterative approach to select the most appropriate area for species and habitat protection, this concept of complementarity offers the advantage of efficiency and explicitness over other methods (Margules *et al.* 1994). Kingston (2001) identified three areas on Pitcairn Island which would provide refuges for most of the islands threatened species as well as providing suitable habitat into which other rare species could be introduced. To date these reserves at Tautama, Big Ridge and Down rope have not been set aside as reserves by the government. However, Wright and Lees (1996) point out that the models of national park or reserves are unlikely to be appropriate to many Pacific Island cultures, mainly because of the long standing cultural relationship between people and natural resources, and complex patterns of land ownership. Denial of access and the ability to utilise resources are unlikely to find much favour with local people. The aim of reserves should be to conserve local forest resources in conjunction with the participation of local people (Waldren 2002).

Species recovery plans usually aim to increase the number of individuals and broaden the genepool of a particular threatened taxon. A criticism of species recovery programmes is that they

may ignore ecological interactions at the community level by focussing on recovery of a single species. Conversely simply setting aside protected areas may not always maintain sufficient individuals of a threatened taxon to maintain a viable population. Integrated approaches are required which utilise a full range of available techniques and which are mindful of the hierarchical nature of biological diversity (Waldren 2002).

The integration of development, social cohesion, increased management of biodiversity (habitats and species) and in particular invasive species into the future on islands is a major challenge. The Millennium Ecosystem Report: Island Systems (1995) highlights the fact that close links that are required between all of the above to ensure islands are economically, socially and ecologically resistant and self-sufficient.

1.3 Introduction to the Pitcairn Island Group

1.3.1 Pacific Oceanic Islands

Four major regions have been delimited in the Pacific; they are Melanesia, New Zealand, Micronesia and Polynesia. Melanesia extends northwards from New Guinea and southwards to New Caledonia and Fiji. The flora is of these islands is considered an extension to the rich Indo-Malaysian flora. They generally have large land areas and large and diverse floras (Mueller-Dombois & Fosberg 1998). New Zealand had land connections with Australia and Antarctica in the past, an Australian element in the flora is prominent but because these connections were in the remote past it is depauperate in species and endemism is high, and in this characteristic the New Zealand flora resembles those of more insular islands. The flora of the Micronesian region has relationships with Indonesia and Asia as well as New Guinea and Australia, and a Pacific strand flora is well represented on these islands. The Polynesian region to which the Pitcairn group of islands belong have a predominately Indo-Pacific flora (Mueller-Dombois & Fosberg 1998). A more recent assessment by Kingston *et al.* (2003) found that Pitcairn is dominated by species with pacific, Polynesian and endemic distributions.

1.3.2 The Pitcairn Island Group

The Pitcairn Island group comprises of four islands located in the South-Central Pacific. The islands are part of the United Kingdom Overseas Territories, and are exceptionally remote, lying at the south-eastern extremity of the central Polynesian islands south of the Tropic of Capricorn (1570km West of Easter Island; 5350km North-East of New Zealand, Figure1.1). The group consists of two low atolls, Oeno and Ducie the latter is the most southerly atoll on earth, the raised atoll Henderson (a World Heritage Site) and a high volcanic island Pitcairn.

The estimated total land area of the Pitcairn group is 4516ha (Pitcairn 660ha, Henderson 3720ha, Oeno 62ha & Ducie 74ha) (Bell & Bell, 1998, Waldren *et al.* 1995a). The climate of the

Pitcairn Islands is sub-tropical with mean annual rainfall of approximately 1716mm but with considerable annual variation. The temperature ranges from 17°C to 28°C in summer and from 13°C to 23°C in winter, the winter period is also wetter and windier than the summer period (Spencer 1995).

Much of the biological interest in the group has centred on Henderson, designated a World Heritage Site for its unique and relatively undisturbed biota (Benton & Spencer 1995; Brooke *et al.* 2004). However, much of the plant conservation interest has centred on Pitcairn (Waldren *et al.* 1995 a & b; Kingston 2001; Kingston & Waldren 2003 & 2005), the only inhabited island in the group and the home of the descendants of the *'Bounty'* mutineers.

Henderson is a raised coral island (*makatea*) and is thought to be the Pacific's best remaining example of an elevated coral atoll ecosystem and thus is of outstanding natural value (Fosberg *et al.* 1983). Its remoteness and inhospitable terrain have protected it from human modification and its high elevation (30m) from inundation; as a result a diverse and unique flora and fauna have arisen (Fosberg *et al.* 1983 Benton & Spenser 1995, Waldren *et al.* 1995 a & b). Henderson is currently uninhabited, but during Polynesian times (1220-1650 AD) up to 100 people may have resided on the island (Weisler 1995; Diamond 2005). The plants they introduced and cultivated mostly became extinct soon after the people though some plants remain from that time such as Miro (*Thespesia populnea*) and Candlenut (*Aleurites moluccana*) (Waldren *et al.* 1999a). Henderson is thought to be the only example of a Pacific Island where the present vegetation and fauna mostly reflect the native condition (Göthesson 1997; Oldfield 1999).

Oeno and Ducie are low uninhabited coral atolls with maximum elevations of 1-2m (Waldren *et al.* 1999a). Oeno consists of two small islets which can join temporarily (Sandy Islet & Woody Islet) in a shallow lagoon. The overall area is 15 km^2 with 0.62km^2 of emergent land (Göthesson 1997). Ducie is the smallest of the islands in the group with an overall area of 6.4km^2 of which 0.74km^2 is emergent land rising 1-2m above sea level. The emergent land on Ducie consists of four islets (Arcadia Islet, Edwards Islet, Pandora Islet & Westward Islet) fringing a central lagoon. The land flora and fauna of both are depauperate as the habitats are semi-arid, saline and prone to over-wash in high seas (Waldren *et al.* 1999a).

1.3.3 Pitcairn Island

Pitcairn Island is a small (4 x 2km^2) relatively young (0.75 – 1 million years ago), high volcanic island with steep slopes and a maximum altitude of 329m (Waldren *et al.* 1995a). Pitcairn itself has a wide variety of habitats which consists of rugged sea cliffs on the north and south coasts, scrub and eroding slopes, ridge vegetation, invaded *Syzygium jambos* forest, *Homalium taypau* forest, *Pandanus tectorius* forest and *Meterosideros collina* forest (Kingston & Waldren 2003). It was estimated by Kingston & Waldren (2005) that 10% of the island comprises of level ground, the rest of the island consisting of slopes which range mostly from 20-45° angles with even steeper slopes in the uninhabited valleys.

The current population of Pitcairn is approximately 50, all of whom reside in one settlement, Adamstown. There is, however, evidence of extensive occupation during Polynesian times when local stone was quarried and exported throughout South Eastern Polynesia. Polynesian occupation of the island had ceased by the time the Bounty Mutineers arrived in 1790 (Weisler 1995, Diamond 2005). Currently the main employment on Pitcairn is in local government and community services. Supplementary income is provided by the sale of wood carvings or curios to passing cruise ships, and to a lesser extent by mail order (Oldfield 1999). Access to the Pitcairn islands group requires a licence issued by the Governor, through the Pitcairn Island Administration office in Auckland in consultation with the Island Council (Brooke *et al.* 2004).

Figure 1.1: The Pitcairn Islands Group

1.3.4 The flora and vegetation of Pitcairn Island

A flora consists of both a list and a description of plants found in a particular area. A baseline flora is essential as it provides the basis for all plant related research (Prance 1998). Vegetation, in contrast to flora, refers to species assemblages and the plant cover occupying an area (Muller – Dombois 1998). Small attempts were made to in some way record and classify the flora of Pitcairn island during the 1800's and 1900's but these investigations were often limited to short notes made by scientists who happened to be passing or visiting for a brief period, and whose aims and interests were more often not solely the flora of Pitcairn (Fosberg 1992; Muller-Dombois & Fosberg 1998). Some of the information was also based on herbarium studies and correspondence with islanders (Göthesson 1997). In 1995a Waldren *et al.* published a conservation assessment of the flora of the Pitcairn Island group and in that paper they noted the need for an urgent floristic and vegetation study of Pitcairn Island to be undertaken. With that aim in mind an intensive three month study into the flora and vegetation communities of Pitcairn Island was undertaken by Dr. Naomi Kingston and Dr. Steve Waldren (Kingston 2001; Kingston & Waldren 2005) in 1997. The

flora was found to consist of 81 native plant species and 250 introduced species of vascular plants including 11 species endemic to the island (13.6% of the flora) and two possible endemics (Table 1.1). Eighteen species were found to be threatened globally and 51 species threatened on Pitcairn (Kingston 2001).

Table 1.1: Endemic plant species found on Pitcairn Island (Kingston 2001)

Family	Genus & species
Malvaceae	*Abutilon pitcairnense*
Marattiaceae	*Angiopteris chauliodonta*
Asteraceae	*Bidens mathewsii*
Rubiaceae	*Coprosma benefica*
Aspidiaceae	*Ctenitis cumingii*
Aspidiaceae	*Lastreopsis* c.f. *pacifica*▲
Euphorbiaceae	*Glochidion comitum*
Euphorbiaceae	*Glochidion pitcairnense**
Haloragaceae	*Haloragis* sp. ▲
Flacourtiaceae	*Homalium taypau*
Myrsinaceae	*Myrsine* aff. *niauensis*
Peperomiaceae	*Peperomia pitcairnensis*
Peperomiaceae	*Peperomia sp.*

*endemic to Pitcairn and Henderson Islands

▲Possibly not endemic, most likely to be recent colonist (S. Waldren *pers.comm.*)

The small flora was found to be largely derived from south-eastern Polynesia with the low number of species found a result of the geographical remoteness, the young geological age (<1 million years) and the small size of the island (Kingston & Waldren 2005). The main threat to the biodiversity on Pitcairn Island was found to be widespread alien species such as *Lantana camara* and *Syzygium jambos* (Kingston 2001). Large areas of the island are covered with weedy shrub vegetation and monospecific stands of *Syzygium jambos* (Kingston & Waldren 2003). The spread of these and other invasive species was found to be directly affecting thirty-five native plant species (Kingston & Waldren 2005). Other threats affecting the native flora were clearance of native forest, small population sizes, restricted distribution, erosion, and exploitation (Kingston & Waldren 2005).

1.4 Invasive exotic plant species in Island ecosystems

Islands are highly vulnerable to disturbances associated with human activities, the most serious of which are the introduction and release of grazing mammals and alien plants. Island biotas are adapted to ecosystems free from human disturbance and this feature alone makes them more

susceptible to invasion (Mueller-Dombois & Loope 1990). This observation that islands are more susceptible to invasion by continental plants has been noted since the 1850's. Darwin (1859), Hooker (1867) and Wallace (1895) remarked on the susceptibility of islands and were pessimistic even then about the future survival of island biotas (Mueller-Dombois & Loope 1990). The reasons why islands are more susceptible to invasion are thought to be their general species poverty in comparison to mainland sites (Elton 1958; Simberloff 1995) and the fact that one or two species often overwhelmingly dominate in island forests, for example, *Meterosideros polymorpha* and *Acacia koa* dominate Hawaiian forest (Mueller-Dombois & Loope 1990), and *Pisonia grandis* dominates much of the vegetation of Henderson (Waldren *et al.,* 1995). A survey of the literature relating to invasive species for 184 sites across the globe by Lonsdale (1999) revealed that islands were indeed more invaded than mainland sites, this however, was thought was not because of their low species richness but because they are more invasible or suffer from a higher propagule pressure (Lonsdale 1999: Lockwood *et al.* 2005).

Invasion on tropical islands was also thought to be greater by comparison to islands in temperate climates (Brockie *et al.* 1998). Lonsdale (1999) refuted this and discovered latitude had no influence on the numbers of invasive species on islands though it did have on mainland sites. While the debate continues on the reasons why islands are more susceptible to alien invasive species, the fact remains that alien species are the main cause of species extinctions on islands (Clout & Veitch 2002). To deal with the constant threat of increasing introductions, Daehler *et al.* (2003) has developed a specific risk assessment protocol for screening potential invasive exotic plant species for the Pacific Islands before import, based on the Australian and New Zealand risk assessment protocols. However, increases in human visitors to islands and predicted climate change are more difficult challenges, which are impossible to address without social and political will (Daehler *et al.,* 2003).

1.4.1 Invasive species case study: *Syzygium jambos* (L.) Alston

Syzygium jambos (Roseapple Tree) is native to S. E. Asia and was distributed from there to many parts of the world for timber and fruit cultivation, and it has subsequently become naturalized and invasive throughout the pan-tropics. Reports of *Syzygium jambos* have described it in "thickets" and "solid stands" in Southern Mexico, Peru, Guatemala, areas of West Africa (Morton 1987; Djipa 2000) and Australia (Csurhes & Edwards 1998) (Figure 1.2).

Syzygium jambos has become especially troublesome on islands with reports on its invasive nature from Jamaica, Bermuda and the West Indies (Morton 1987); Puerto Rico (Brown *et al.* 2006); Galápagos Islands, Hawaii, Mauritius, La Reunion, Seychelles (Lawesson 1990); Fiji (Smith 1985); Cook Islands (Space & Flynn 2002); Zanzibar and Pemba (Morton 1987); Micronesia-Pohnpei (Fosberg & Oliver 1979); French Polynesia-Society Islands (Welsh 1998) and Pitcairn Island (St. John 1987; Florence *et al.* 1995; Waldren *et al.* 1995; Göthesson 1997; Kingston & Waldren 2003; Kingston *et al.* 2004, Kingston & Waldren 2005).

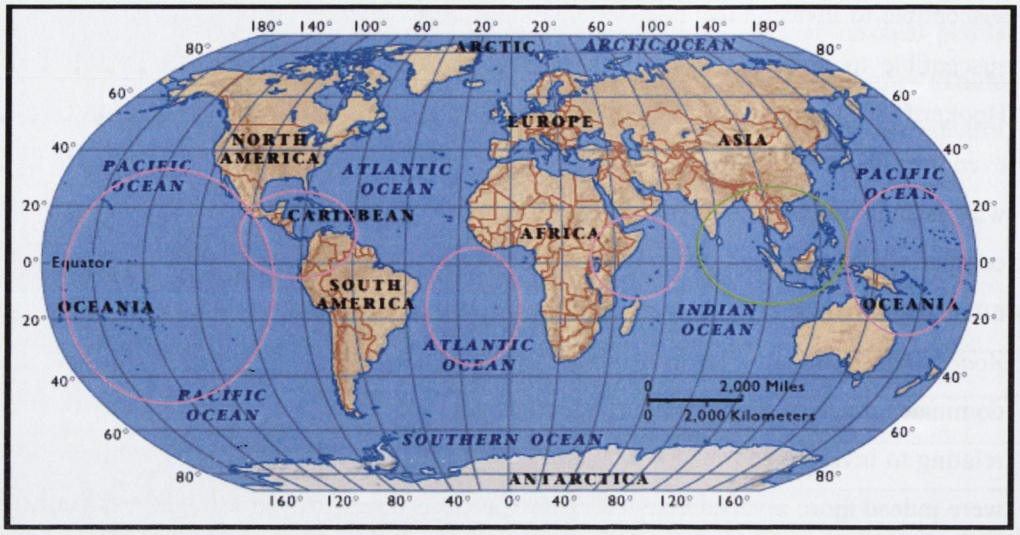


Figure 1.2 *Syzygium jambos* global distribution with native distribution in green and invaded distribution in pink. (Worldmaphttp://go.hrw.com/atlas/norm_htm/world.htm)

Syzygium jambos can flourish in the lowlands of tropical and sub-tropical climates. It is known to naturalise from sea-level up to 915m in Jamaica, from sea-level up to 1,200m in Hawaii; up to 2,300m in Equador and to 1,350m in its native range in S.E. Asia (Morton 1987). In its native habitat, it does best in riparian habitats along the banks of streams and rivers (Morton 1987), and in some invaded habitats, for example in Puerto Rico, it is also known to dominate in riparian habitats (Brown *et al.* 2006). It was however, not exclusively riparian in Puerto Rico and could become dominant in old pasture and abandoned plantations, and was also capable of invading closed canopy native forest (Brown *et al.* 2006). *Syzygium jambos* was found to occur in forest sites of all age stands (5-80 years) in Puerto Rico and highest densities were found in older sites (Aide *et al* 2000). The large seed of *Syzygium jambos* produces a large seedling that can survive and grow in shaded understory (Horvitz *et al.* 1998). In native forest on Santa Cruz (áGalápagos Islands) it was observed to "crowd out" native trees (Kastdalen 1982) and spread into *Scalesia* forest on San Cristóbel, Santa Cruz and Isabela-Galápagos Islands (Hamann 1984).

Syzygium jambos is known to be highly shade tolerant and long lived and can tolerate both damp and semi-arid conditions. The generalist ecological and habitat requirements of allow it to invade in a myriad of habitats as it appeared not to be limited by land use history, soil or topographic factors (Brown *et al.* 2006).

1.4.2 Taxonomy of *Syzygium jambos*

Syzygium jambos (L.) Alston (syn. *Eugenia jambos* L.; *Jambosa jambos* Millsp. *Jambosa vulgaris* D.C.; *Caryophyllus jambos* Stokes) is a member of the Myrtaceae family (Figure 1.3). The Myrtaceae are a mostly southern hemisphere family containing between 132 and 150 genera and between 3,675 and 3,900 species (Mabberley 1997). The two most confused genera in the

Myrtaceae family are *Eugenia* and *Syzygium*. Some authors have advocated distinguishable characteristics between them based on calyx, stamens and anthers (Johnson & Briggs 1984), while others have grouped both genera into a single enormous genera *Eugenia* (Kochummen 1995). The genus *Jambosa* was reduced to synonymy with *Syzygium* by Alston (1931).

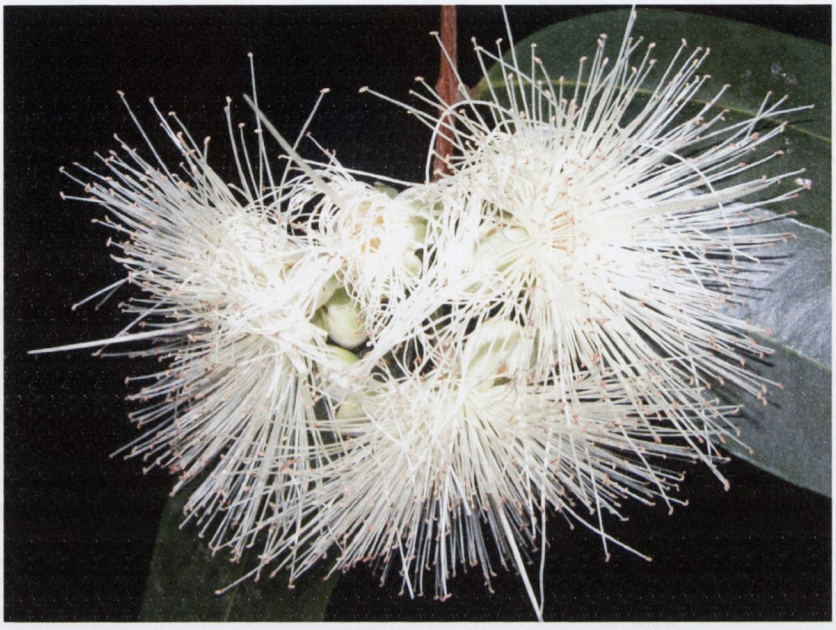


Figure 1.3: *Syzygium jambos* (Myrtaceae) (Photo by S. Waldren)

The current view is to ignore the differences between *Eugenia* and *Syzygium* and not accept *Jambosa* as a genus or subgenus (Parnell 1999). However, a numerical analysis of Thailand's members of the *Eugenia-Syzygium* Group, in which *Syzygium jambos* was included, revealed that *Syzygium jambos* was included within a distinct phylogenetic group and that this group warrants at least status at sectional level (*Syzygium* sect. *Jambosa)* or even perhaps at genus level with the genus *Jambosa* re-installed (Parnell 1999).

1.4.3 Morphological description of *Syzygium jambos*

Syzygium jambos is a fast growing evergreen tree 10±m tall with an extensive root system and wide crown supported by a trunk 20-40±cm in diameter. The leaves are opposite, simple, coriaceous, shiny, and lanceolate, acuminate, 15±cm long and 4±cm wide. The venation is feather-like with a prominent intra-marginal vein 2±mm within the margin with a petiole 7±mm long (Göthesson, 1997).

Flowers are white to pale yellow held in terminal "pompom like" cymes, 7.6±cm in diameter. The corolla consists of 4 petals 12mm long, with numerous stamens 20mm which are long and prominent. The ovary and fruit is inferior. The calyx is generally 13±mm long, with 4 persistent lobes and 6±mm long. Fruit is globose, subspherical to pear shaped and edible 2.3-5±cm in diameter and can be white, yellowish reddish or pink in colour (Göthesson, 1997).

The centre of the fruit is hollow with 1-4 (usually 2) brown, rough-coated, medium-hard, round-shaped seed (1-1.6cm thick), which loosen from the inner wall as the fruit matures. The seeds are polyembryonic and produce one to three sprouts which can grow rapidly and fruit within four years (Morton 1987).

1.4.4 Traditional and current uses of *Syzygium jambos*

The fruit of Syzygium *jambos* were popular in the past and they were eaten raw or made into preserves and jellies and when distilled the fruits yield good quality rosewater. The fruit is however easily bruised and considered insipid to taste, and a mature tree yields only 2kg of fruit in a season, which is considered a small return from a tree that occupies so much space; not surprisingly it has fallen out of favour (Morton 1987).

It is an excellent source of firewood, and has been used for amenity plantings as a windbreak (Lycos Website 2005; Göthesson 1997). The large and showy flowers are a rich source of nectar for honeybees and honey production on Pitcairn Island is currently largely dependent on *Syzygium jambos* which produces a rich amber-coloured honey (*pers. obs). Syzygium jambos* uses also include medicinal and industrial applications. The fruits have been used as a tonic for the brain and liver. A sweetened preparation of the flowers is believed to reduce fever and roasted, powered seeds and teas have been used to benefit to diabetics (Morton 1987, Teixeira *et al.* 1990). Tannin and a brown dye can be extracted from the bark. Hydrocyanic acid has been found in the roots, stems and leaves and Jambosine (an alkaloid) is found in both the bark and roots (Morton 1987). Antimicrobial activity has been reported from bark extracts (Djipa *et al.* 2000). Essential oil can be distilled from the leaves (Morton 1987) and volatiles for possible use in the food industry were identified by Guedes *et al.* (2004).

1.5 Aims of study

The aims of this study were to provide an integrated approach to the conservation of the biodiversity of Pitcairn Island. The first step was to assess the effects of the invasive alien species *Syzygium jambos* on the diversity and ecology of the island (Chapter 2). The second step was to develop techniques to control *Syzygium jambos* using practical and pragmatic methods to achieve both a cost effective and successful method of control (Chapter 3). The third step was to rehabilitate the native forest community on the island taking into account the services that the local human community require from forest ecosystems on the island (Chapter 4). The final step was to make a conservation assessment of the species that are most threatened and in danger of extinction or in need of further study to assess their status on the island (Chapter 5). The results of these investigations were combined (Chapter 6) to outline management techniques and recommendations for dealing with the invasive species *Syzygium jambos*, forest rehabilitation and for monitoring and maintaining threatened plant populations on the island for the future.

The effects of alien invasive species on biodiversity have been described as "*immense, insidious and usually irreversible*"

(IUCN 2000)

Chapter 2

Effects of *Syzygium jambos* (L.) Alston on the diversity and soil ecology on Pitcairn Island

2.1 Introduction

The extent of the effect of non-native species on vegetation composition in tropical forests can depend on whether the non-native species in question is transient (Lugo 2004) or persistent (Brown *et al.* 2006). The appearance of transient non-native species in a disturbed forest can often eventually lead to the recovery of mature native forest (Terbourgh *et al.* 1996; Grau *et al.* 1997). However, in many cases non-native species can limit native plant regeneration (Lichstein *et al.* 2004) and if the non-native species endure, they can cause a fundamental shift in the forest diversity and community structure (Brown *et al.* 2006).

2.1.1. The effects of non-native invasive species

Invasive non-native plant species often affect all components of an environment from biodiversity (Brown & Gurevitch 2004) to ecosystem processes (Vitousek & Walker 1989; Dyer & Rice 1999). The effects of invasive species on biodiversity have been described as "immense, insidious and usually irreversible" (IUCN 2000). Many non-native invasive species are known to completely alter the nutrient cycling and hydrology in native ecosystems and diminish the abundance or survival of native species (Mack *et al.* 1999).

Non–native invasive plants are considered extremely damaging on islands, despite the fact that islands have generally low biodiversity, because a high proportion of native species found on islands are endemic species. The high numbers of endemic plant species found on islands have led many of them to be described as "botanical wonderlands"; for example, the Canary Islands flora consists of approximately 500 endemic plant species which consist of 40% of the total flora (Francisco-Ortega *et al.* 2000, Wade 2005). The ecosystems of all the Pacific islands have been greatly affected by non-native invasive plant species (Loope & Mueller-Dombois 1989). The Hawaiian Islands currently have 4,600 introduced exotic plant species, three times the number of indigenous species (St. John 1973) and 1000 of these have become naturalised (Wagner *et al.* 1999).

The Galapagos Islands hold 176 endemic species (32% of its flora), with 7 endemic genera, and 45% of the total flora consists of non-native plant species. The number of non-native species was found to correlate strongly with human population increases (Mauchamp 1997).

In French Polynesia the percentage of non-native introduced plants stands at 37%, in New Caledonia the figure is 13% and on the Solomon Islands 6% (Vitousek *et al.* 1996; Given 1992).

Despite in some cases, the large percentage of plants introduced on these islands, only 2% of them are aggressive and compete with native and endemic plants (Mauchamp 1997).

A large number (around 250) of exotic plant species have been introduced to Pitcairn Island (Kingston & Waldren 2005). The majority of alien plants introduced to the island have not become widespread, but some have become troublesome, for example, Lantana *camara* and *Sorghum sudanense* (Kingston & Waldren 2003). It is currently estimated that 40% of the island is covered by stands of *Lantana camara* and *Syzygium jambos* (Kingston & Waldren 2005). The spread of invasive species on Pitcairn was found to be affecting 35 native taxa or 43.2% of the flora (Kingston & Waldren 2005).

2.1.2 Native Forest Communities on Pitcairn Island

Kingston & Waldren carried out an assessment of the flora and vegetation of Pitcairn Island in 1997 (Kingston 2001). They used 10x10m^2 quadrats for their assessment and identified fourteen different plant communities on Pitcairn Island. Six of the fourteen communities identified were forest communities: *Syzygium jambos* invaded forest, *Homalium taypau* forest, *Pandanus tectorius* forest, *Meterosideros collina* forest, mixed forest and dry mixed forest (Kingston & Waldren 2003). Native forests communities on Pitcairn Island were found to be most similar to the categories of montane rain forests and cloud forest as defined by Muller-Dombois and Fosberg (1998) for the tropical Pacific (Kingston 2001; Kingston & Waldren 2003) (Figure 2.1)

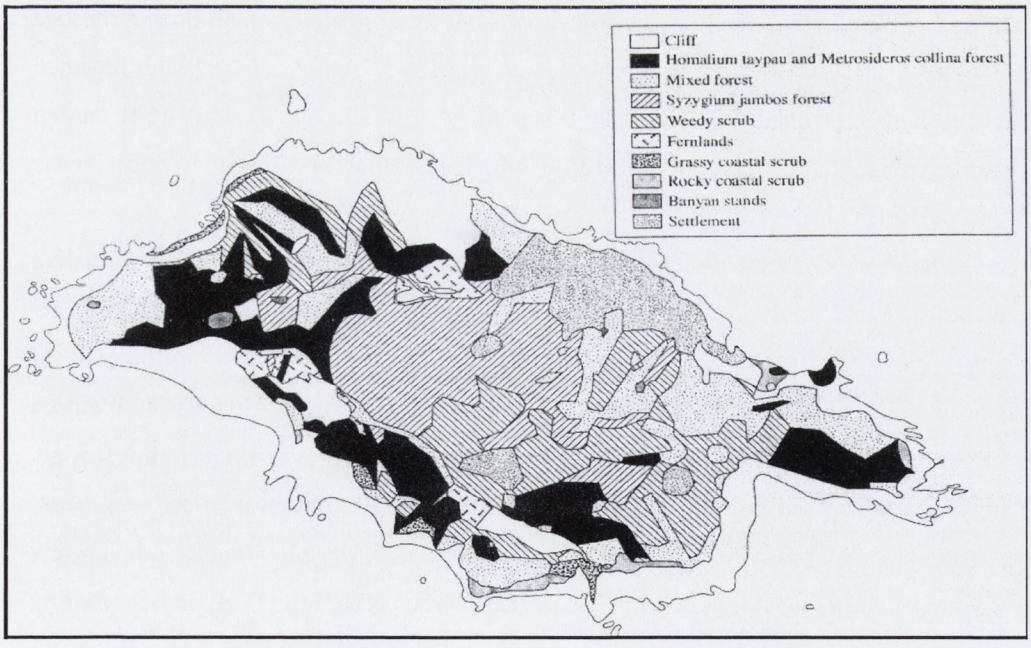


Figure 2.1 Map showing distribution of plant communities across Pitcairn with *Syzygium jambos* forest dominating the vegetation in the centre of the island (Kingston & Waldren 2003).

Meterosideros collina forest was found occurring in the higher parts of the island and in southern and western valleys. *Meterosideros collina* forest was found to have a characteristically rich pteridophyte and epiphytic flora. This forest type had a low-density canopy and with small

leaves, it allowed for greater light penetration to the forest floor. *Meterosideros collina* and the species found in association with it, such as, *Homalium taypau*, *Cyathea medullaris*, *Xylosma suaveolens* and *Glochidion* spp. are thought to represent the native forest of the island prior to human settlement (Kingston & Waldren 2003).

Homalium taypau forest was found in scattered patches across the island and was found to integrate with *Meterosideros collina* at high altitudes and *Pandanus tectorius* forest in the southern part of the island. Where pure stands of *Homalium taypau* exist on the island these stands were thought to represent stands previously managed for timber and thus could not be considered natural vegetation. Characteristically these pure stands had a species poor ground flora with weedy native fern species dominating the understory (Kingston & Waldren 2003).

Pandanus tectorius forest also forms part of a mixed forest group, which includes species such as *Hernandia sonora*, *Celtis pacifica*, *Hibiscus tiliaceus* and *Glochidion* spp. among others. These forest communities were found to have soils with high loss on ignition and percentage litter cover values when compared with the other vegetation types found on the island. The other types found, included, *Syzygium jambos* forest, ridge vegetation, scrub and eroding slope vegetation and north and south coastal vegetation communities. All of the fourteen plant communities found on Pitcairn Island contained large proportions of introduced species (Kingston & Waldren 2003).

2.1.3 *Syzygium jambos* invaded forest on Pitcairn Island

Syzygium jambos was originally introduced to Pitcairn Island from Norfolk Island during the late 19th century for fuel wood, as it is a fast and vigorous growing tree, capable of surviving repeated harvest. When the human population of the island started to decline after World War II garden plots were abandoned and *Syzygium jambos* spread into these areas and began to form dense thickets (Göthesson 1997).

Syzygium jambos has been directly implicated in causing the severe decline of native Pitcairn biodiversity (Waldren 1995; Preece 1995; Kingston & Waldren 2003, Kingston *et al.* 2004; Kingston & Waldren, 2005). *Syzygium jambos* dominates particularly in the north centre of the island, forming the largest area of forest cover on the island (0.17km^2) (Kingston & Waldren 2003) (Figure 2.1). Two areas within *Syzygium jambos* forest, Brown's Water and Pulau Bridge, contain the important fern (*Lastreopsis* c.f. *pacifica*), though it is thought that this fern is a recent colonist to the island (see Chapter 5). In general the dense stands of *Syzygium jambos* contain few native species and lack a ground flora due to the shade cast by dense canopy. The shallow rooting nature of *Syzygium jambos* is thought to contribute to soil erosion on the island by increasing water run-off and decreasing water absorption into the soil. *Syzygium jambos* was also deemed to be one of the main threats to, and accounts for the population reduction of one of the islands critically endangered endemic ferns, *Angiopteris chauliodonta* (Kingston *et al.* 2004). In a conservation appraisal of the island, Kingston & Waldren (2005) recommended the control of widespread alien species and the prevention of further spread of *Syzygium jambos* into native forest.

Syzygium jambos dominated woodland has, however, been of use and benefit to the human population of Pitcairn Island. The islanders have over the years used *Syzygium jambos* as a source of fuelwood, and building timber. Göthesson (1997) highlights the fact that without *Syzygium jambos* woodland providing fuelwood and timber, many of the remaining stands of native trees, such as *Meterosideros collina* & *Homalium taypau* would have long since disappeared. In more recent times, a thriving honey production industry has grown on the island, and given the extent of *Syzygium jambos* forest, it has become an important source of pollen and nectar for introduced honeybees. The introduction of honeybees (*Apis mellifera*) can facilitate the spread of an invasive species and the interaction between introduced plants and pollinators can further threatens ecological communities (Stout *et al.* 2002). The advantages and disadvantages of invasive *Syzygium jambos* woodland to Pitcairn Island biodiversity and the islanders are outlined in Table 2.1.

Table 2.1 Impacts of the invasive plant species *Syzygium jambos* on Pitcairn Island

Impact of *Syzygium jambos* of Pitcairn islands Ecosystems	**Impact: positive/negative**	**References**
Main threat to native plant biodiversity	Negative	Waldren *et al.* 1995
Causing decline in endemic snail population	Negative	Preece 1995
Threatening to invade and destroy remnant native forest stands	Negative	Florence *et al.* 1995
Causing soil erosion on steep slopes due to lack of ground flora	Negative	Kingston & Waldren 2003; Kingston & Waldren 2005
Population reduction in Endemic fern *Angiopteris chauliodonta.*	Negative	Kingston *et al.* 2004, Kingston & Waldren 2003
The use of *Syzygium jambos* timber as firewood protected the destruction of remnant native forest stands.	Positive	Göthesson 1997
Syzygium jambos flowers extremely important to honey production on the Island	Positive	*Pers. Obs.* (2003,2004 & 2005); Pitcairn Island Produce Co-op (PIPCO); L. Jaques (Commissioner to the Pitcairn Islands)

2.1.4. Soil and invasive plant species

Introduced plants have been used in many instances to prevent soil erosion. Species chosen for erosion prevention are selected for their rapid growth and tenacity, and these features have led to many of them becoming invasive in the introduced area. The following species for example, *Elaeagnus angustifolia* (Russian olive), *Rosa multiflora* (Multiflora rose), *Pueraria lobata* (Kudzu) and *Tamarix ramossima* (Saltcedar) have been used extensively for erosion prevention (Myers & Bazely 2003).

There are reported incidences of invasive plant species altering soil properties. The two most common soil properties to be altered are nitrogen levels and salinity. Nitrogen-fixing invasive

plants can increase nitrogen levels in nitrogen-limited environments, and can cause native flora displacement. Soil salinity levels can be increased by salt secretion onto leaves, which in turn gets washed off into the soil (Myers & Bazely 2003; Yelenik *et. al* 2004; Vivrette & Muller 1977; Zavaleta 2000). Salt secretion from invasive species such as *Tamarix* and *Mesembryanthemum* can increase soil salinity levels and this, salinity increase, can cause displacement of native flora (Vivrette & Muller 1977; Zavaleta 2000).

Myrica faya (syn. *Morella faya* (Ait.) Wilbur) introduced to Hawaii in the 1800's to help prevent soil erosion on volcanic slopes, increased the nitrogen levels, in what was a nitrogen-limited environment. However, there are no indications that the altered nitrogen levels have affected the native biota (Myers & Bazely 2003). *Acacia saligna,* also with a nitrogen-fixing ability, was found to alter the nitrogen levels in fynbos vegetation in South Africa. Clearing sites invaded with *Acacia saligna* in South Africa caused a wave of secondary invasion by nitrophilous weedy species, thus causing a direct affect on the native biota (Yelenik *et al.* 2004).

Some invasive species affect general soil fertility levels. *Lantana camara,* is one of the most serious invasive woody weeds in the world (Cronk & Fuller 1995; Rejmánek & Richardson 1996; Binggeli *et. al* 1998). *Lantana camara* has also been reported to improve the general soil fertility (Ghisalberti 2000) and long term additions of *Lantana camara* biomass to rice and wheat fields increased yields by 22-29% (Sharma & Verma 2000). On Pitcairn Island, sites with *Lantana camara* are traditionally the preferred sites for opening new vegetable plots (*pers. obs*. 2003-2005; T. Christian *pers. comm*. to S. Waldren 1997).

Syzygium jambos has a preference for deep loamy soil but it is not exacting in this requirement as it can also grow on sand and limestone with little organic matter (Morton, 1987). Soils with large pools of phosphorous, calcium and potassium had dominant stands of *Syzygium jambos* in Puerto Rico (Brown *et al.* 2006). Analysis of soils in *Syzygium jambos*-dominated habitat, in the Ludquillo Mountains Puerto Rico, also concluded that *Syzygium jambos* can establish in variable soils (Brown *et al.* 2006). Although in that study it remained unclear as to whether *Syzygium jambos* invasion was a cause or consequence of the observed soil conditions (Brown *et al.* 2006)

2.1.5 The forest soils of Pitcairn Island

The first soil survey of Pitcairn Island was carried out by Twyford (1958), seventeen samples were analysed from eight sites on the island. His investigations were prompted by the results of samples collected by an Administrative Officer Mr. Clayton in 1954, who found abnormally high phosphate levels in Pitcairn soil, which suggested there might be a payable phosphate deposit on Pitcairn Island. The soil analysis results obtained from Twyford's study indicated that soil fertility was generally high, organic matter was low and he attributed the high phosphate levels to underlying basaltic rock and found no evidence of seabird guano deposits.

One of the eight sites investigated by Twyford (1958) was at Flatlands where *Syzygium jambos* now dominates. In 1958 this site consisted of gardens, fruit trees and some *Syzygium jambos* thicket. Twyford noted that some of the differences in soil organic matter and nitrogen on Pitcairn were due to plant cover, especially in areas where gardens have been in existence for a long time, and he also noted an increase in soil depth and definite evidence of leaching in soils under the invasive species *Lantana camara* and *Syzygium jambos* (Twyford 1958) at the Flatland site. Investigations into pH and organic content were carried out for 83 sites across the island in the different vegetation communities (Kingston 2001). Soils investigated showed higher pH values in native woodland communities when compared with scrub, coastal and *Syzygium jambos* woodland communities. The lower pH values found in scrub, coastal and *Syzygium jambos* woodland communities was thought to be as a result of excessive erosion (Kingston 2001). High values for organic content were also found for *Syzygium jambos* forest (Kingston & Waldren 2003).

2.1.6 Data analysis techniques in vegetation studies

Data analysis methods in vegetation studies are used to define "the frightening and unknown mass of green" (Randall 1978). The building blocks of vegetation are plant species, describing their abundance and the environment they grow in a key aim in vegetation analysis. Information on vegetation is important as its aids biological conservation and management and provides a basis for monitoring current management practices and can provide a basis for prediction of future vegetation changes (Kent & Coker 1992). Vegetation data are generally recorded in two-way (quadrat by species) matrix, these data are multivariate, each species or quadrat added to the data set adds extra variation. The two main types of vegetation data collected are nominal (categorisation without numerical value as in presence absence data (0 or 1) and ordinal where data are placed in a rank along a continuum for example, the Domin scale, Table 2.3.

General statistics and tests

Normality Test

Many statistical tests make assumptions that the data have been collected by random sampling and that the data collected are normally distributed. In the normal distribution, 68% of the observations fall within the standard deviation of the mean, 95% of the observations fall within twice the standard deviation of the mean and 99.7% of the observations are within three times the standard deviation of the mean (Moore & McCabe 2003).

A test of data is required before deciding on which statistics can be used to analyze. Cochran's (1951) test of heterogeneity of variances is considered the most useful for ecological studies (Underwood 1997). Cochran's C is a test statistic of the ratio of the largest deviation (S_i) to the sum of sample variances ($\sum S_i$) and is calculated using the formula outlined in Equation 2.1.

$$\text{Cochran's C} = \frac{\text{largest } S_i^2}{\sum S_i^2}$$

Equation 2.1 Cochran's C Test (Cochran 1951)

If Cochran's test is significant, the data are not suitable for any ensuring analysis of variance and only tests which are capable of dealing with heterogeneity of variances data can be used. When the data can be considered normal (Cochran's test not significant) a range of standard analysis of variance statistics can be used to interpret data (Underwood 1997).

Correlation

Correlation can be used to assess the strength of a relationship between species and the environment or a specific factor. The result of a correlation is a statistic lying between -1.00 through 0 and +1.00 which describes the degree of relationship between the two variables in question. The most commonly used parametric correlation coefficient is Pearson's product moment correlation coefficient (Equation 2.2). The non-parametric equivalents are Kendall's Tau and Spearman Rank correlation coefficients.

$$\mathbf{r} = \frac{\sum x_i y_i - (\sum x_i)(\sum y_i)/n}{\sqrt{[\sum x_i^2 - (\sum x_i)^2/n]}\sqrt{[\sum y_i^2 - (\sum y_i)^2/n]}}$$

r = product-moment correlation coefficient
n=number of pairs of observations
$\sum x_i$= sum of observations on x
$\sum y_i$ = sum of observations on y

Equation 2.2 Pearson's product moment correlation coefficient

The value of Pearson product moment correlation coefficient (r) can then be tested for significance ($t = \mathbf{r}\sqrt{n-2/1-r^2}$). To calculate the non-parametric equivalent; Spearman's rank correlation coefficient ($\mathbf{r_s}$) the data are arranged into ranks from low to high or high to low and once ranks are assigned the difference between each pair of ranks is calculated (d) and squared and used in Equation 2.3

$$r_s = 1 - \frac{6\sum d^2}{n^3 - n}$$

r_s=Spearman rank correlation coefficient
d = difference between paired ranks
n=number of pairs of observations

Equation 2.3 Spearman's rank correlation coefficient

Regression

Regression outlines the nature of the relationship between the variables analysed, if the relationship between the two variables is linear (Equation 2.4) regression analysis allows prediction of one value in terms of the other. As with analysis of variance the statistic used depends on parametric or normal data. Least squares regression is used when the data are parametric.

$$y = a + bx$$

y is the response variable plotted on vertical axis
x is the explanatory variable plotted on the horizontal axis
b= slope of the line (the amount y changes when x increases by one unit
a = intercept i.e. they value of y when x = 0

Equation 2.4 Equation of a line

To fit a least squares line to points on a scatterplot the line which minimises the sum of the squared deviations distance between the actual points and the fitted line (the "error") is the best. Error can be calculated by subtracting the predicted value (on the fitted line) from the observed value or actual data point.

Analysis of Variance

Analysis of variance (ANOVA) compares the variance for several groups. It can be used to assess relationships and variation within and between groups only if the variances of the population are equal and the population is normally distributed. When data are not normally distributed, more suitable tests include the Mann-Whitney U test, comparing 2 samples (Equation 1.6) and Kruskal-Wallis test, comparing more than 2 samples (Equation 1.7). When there is more than one treatment (or factor) in an experiment additional factors are added to the ANOVA and additions for the interaction between each treatment (factor) unless the analysis is nested. The results of ANOVA indicate whether a specific treatment or factor is having an affect on the groups in question but cannot say which groups are different from each other. The ANOVA F statistic only provides the basis for assuming that means are not all the same (Moore & McCabe 2003).

Post Hoc Tests

In order to access which group means are different from ANOVA results, post hoc tests have to be performed. There are many different types of post hoc tests, each of which have their advantages and disadvantages. One used commonly in ecology and in invasive species study (Godefroid *et al.* 2005) is the Student Newman Keuls (SNK) test (Underwood 1997). This test arranges the means of groups in ascending order; the means furthest apart are most likely to be different from each other. The means of each pair are compared using the SNK test statistic **Q** as outlined in Equation 2.5

$$Q_{ij} = \frac{\text{Mean}_i - \text{Mean}_j}{\text{Standard error (SE)}}$$

Equation 2.5 Student Newman Keuls Test Statistic Q

Mann Whitney U test for non-normal data

The Mann Whitney U-test is a non-parametric test which compares the medians of two unmatched samples. The values of observations are converted to their ranks. The ranks of each sample are summed and the test statistic U is calculated. U_1 and U_2 are calculated as $U_1=n_1n_2+n_2(n_2+1)/2-r_2$ and $U_2=n_1n_2+n_1(n_1+1)/2-r_1$, where n_1 is the number of observations in sample 1 and n_2 the number

of observations in sample 2 and r_1 is the sum of ranks in sample 1 and r_2 the sum of ranks in sample 2 (Fowler *et al.* 1998).

$$\text{Mann Whitney U} = \frac{U_1 - U_2}{n_1 n_2}$$

Equation 2.6 Mann Whitney U test

Kruskal-Wallis K test for non-normal data

When there are more than two samples to compare the Kruskal Wallis K test can compare the medians of three or more samples. The observed values are converted to ranks and ni is the samples sizes (i=1,2,..k) for each of the k groups or samples and N is the overall number of samples from all groups. The constants 3 and 12 are particular to this formula. The test statistic K is calculated as in equation 2.7 (Fowler *et. al* 1998).

$$\text{Kruskal Wallis K} = \sum_{i=1}^{k} [R^2/n \times 12 / N(N+1)] - 3(N+1)$$

Equation 2.7 Kruskal-Wallis K test

Vegetation description and analysis

Species Association

Calculations exist for determining species association within vegetation communities. A simple measure of association between species and the level of similarity between quadrats is Chi-square (χ^2) (Equation 2.8). Data are arranged in columns and rows in a contingency table.

$$\chi^2 = \sum \frac{(\text{observed species abundance} - *\text{expected species abundance})^2}{\text{expected species abundance}}$$

[*expected abundance = row total x column total/ no. of samples (n)]

Equation 2.8 Chi-square (x^2)

Ordination

Ordination is the arrangement of samples in relation to each other in terms of their similarity of composition and associated environmental controls (Kent & Coker 1992) in three dimensional space. Direct gradient analysis displays variation in relation to environmental factors by using the environmental data to order the vegetation samples. Indirect ordination examines the variation within the vegetation data initially and interprets it before proceeding to incorporate environmental data. Indirect ordination methods have become the preferred method (Kent & Coker 1992). Two common types of indirect ordination are Detrended Correspondence Analysis (DCA) (Hill & Gausch 1980) and Canonical Correspondence Analysis[1] (CCA) which incorporates correlation and

[1] Not strictly indirect ordination as the axes are constrained by multiple regressions with environmental factors

regression with environmental factors (ter Braak 1988). It has the advantage of not only expressing patterns of variation in floristic composition, but outlines the relationship between species and the environmental variables measured.

On an ordination plot, each point represents a sample the greater the distance between any two points the greater the difference in floristic composition in the sample they represent. Eigenvectors are scores scaled like correlation coefficients from -1 through 0 and to =1, every sample has a eigenvector score, the nearer the score to -1 or +1 the more important that sample or variable is in the data. Eigenvalues on the other hand are values which represent the relative contribution of each component to explanation of the total variation in the data. The development of biplot displays on ordination diagrams has arisen as a result of the using sample scores. The direction of the arrow on diagram indicates the direction in which abundance is increasing for that sample or environmental factor, a long arrow indicates a gradual rate of change and a short arrow rapid change (Kent & Coker 1992). The Sørensen coefficient was originally designed for presence-absence data (Equation 2.9). As compared to Euclidean distance, it retains sensitivity in more heterogeneous data sets and gives less weight to outliers.

Sørensen distance = 1-2W/(A+B)

where W is the sum of shared abundances and A and B are the sums of abundances in individual sample units

Equation 2.9 Sørensen Distance

NMS ordination is a search for a ranking and placement of n entities on k dimensions (axes) that minimizes the stress of the k-dimension. The central computational algorithm, the steepest descent minimization to find minimum stress, is based on Kruskal (1964). To test the robustness of the data, a Monto Carlo randomised test of 100 runs or less can be selected. NMS is considered one of the most defensible techniques during peer review because it depends on only a biological meaningful view of the data, providing the standardisation and similarity co-coefficients that are appropriate to the hypothesis under investigation. It is considered to have enhanced distance preserving properties in that rank order is preserved among sample dissimilarities in the overall rank order of distance (McCune & Grace 2002).

2.1.7 Aims of this study

The main aims of the investigations performed in this chapter were to determine the species composition, plant species diversity, density of stand and soil properties of the invaded *Syzygium jambos* forest community on Pitcairn Island and make comparisons where possible with the native forest communities described by Kingston and Waldren (2003). The specific aims were:

1. To investigate and compare, species composition and species diversity of invaded *Syzygium jambos* forest and compare the results to native forest stands on the island, and other *Syzygium jambos* invaded forests in Puerto Rico (Brown *et al.* 2006).

2. Investigate the soil properties and density of *Syzygium jambos* invaded forest and compare aspects of it to native forest soil properties on Pitcairn Island and other *Syzygium jambos* invaded forest soils.

2.2 Materials and methods

2.2.1 Native forest plot selection

The 10x10m quadrats that were classified by Kingston and Waldren (2003) using two-way indicator species analysis as native woodland communities were selected for inclusion in the study performed (Table 2.2). This amounted to forty-three, 10x10m quadrats from sites in various locations throughout the island (Figure 2.2). DOMIN species values (Kent & Coker 1992), pH and organic matter content results from these quadrats were used for analysis.

Table 2.2 Native woodland community quadrats investigated by Kingston & Waldren (2003) and used for analysis

Homalium taypau forest	*Pandanus tectorius* forest	*Meterosideros collina* forest
13, 15, 17, 28, 34, 35, 40, 42, 78, 82, 83	7, 16, 21, 23, 27, 36, 37, 38, 39, 44, 70, 71, 73, 74, 77	52, 65, 66, 4, 48, 49, 50, 51, 53, 54, 55, 56, 57, 64, 67, 75, 81

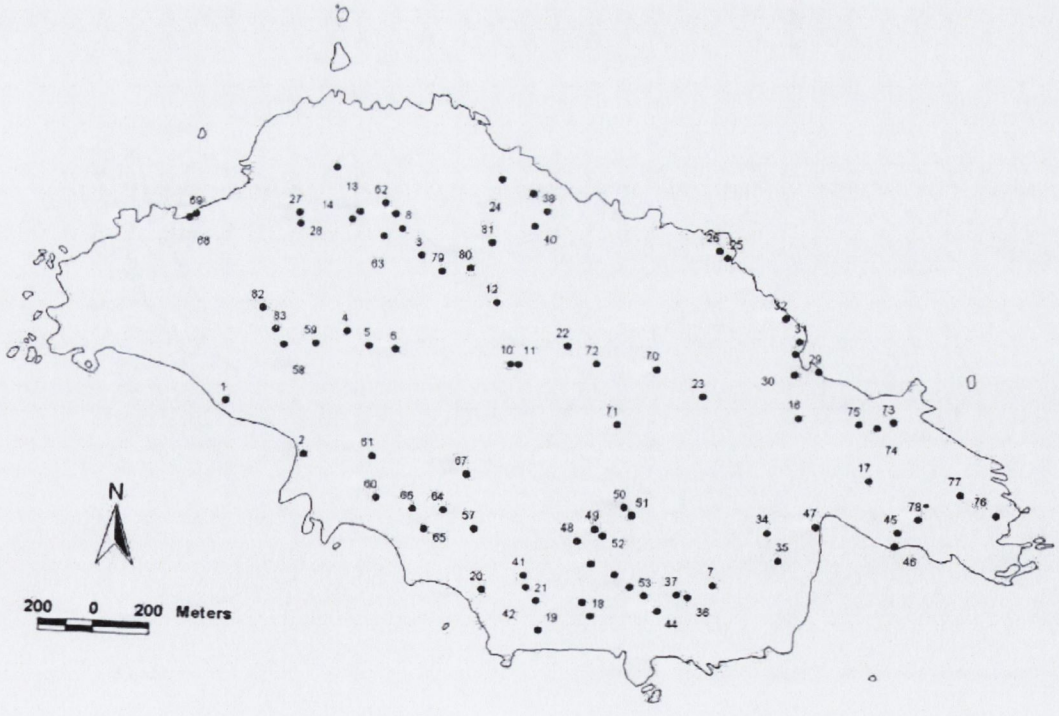


Figure 2.2 Location of 10x10m quadrats investigated by Kingston & Waldren (2003).

2.2.2 *Syzygium jambos* invaded forest plot selection

Twenty-one, (20x20m) permanent plots were selected, with four (10x10m) experimental subplots labelled a, b, c, and d, within each plot. This gave a total of 84 permanent experimental subplots within *Syzygium jambos* invaded forest. The plots were distributed throughout the *Syzygium jambos* forest community as defined by Kingston and Waldren (2003) (Figure 2.3) The Land Court of Pitcairn Island visited and approved all the experimental plots selected, and declared the plots and subplots chosen as government land, thus there were no issues of ownership or interference with experimental treatment. The experimental plots and subplots chosen encompassed the various densities of *Syzygium jambos* invasion in differing island topographies.

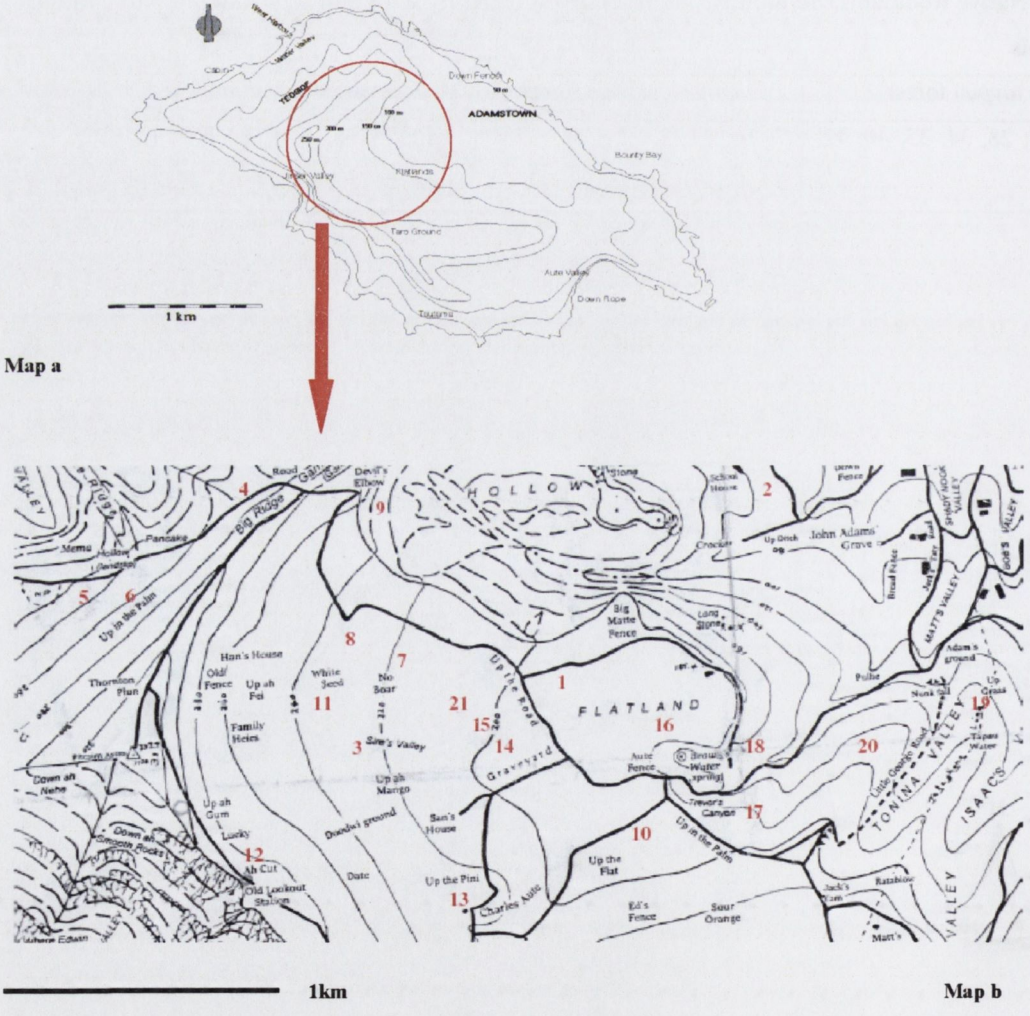


Figure 2.3 Permanent experimental plot locations within *Syzygium jambos* dominated forest (map a) and a detailed plot location map (map b) with experimental plot numbers marked in red.

2.2.3 Vegetation Data Collection

Vegetation data

Floristic vegetation description methods were used to record the vegetation in each of the experimental plots in *Syzygium jambos* dominated forest. Vascular plants were recorded from each of the $10x10m^2$ plots nested within the $20x20m^2$ sites. Species present in the plots were identified and their associated abundance values were recorded using the DOMIN scale (Kent & Coker 1992) (Table 2.3).

Table 2.3. The abundance ranking for taxa with the recorded values in the DOMIN Scale

Value	Cover
+	Single individual
1	1-2 individuals
2	<1%
3	1-4%
4	6-10%
5	11-25%
6	26-33%
7	34-50%
8	51-75%
9	76-90%
10	91-100%

Domin cover values for unvegetated surfaces were also recorded using the following categories: **BS**= bare soil; **SG**=stone; **LL**=leaf litter; **BD**=bedrock; **S**=Sand; **WD**=Woody debris; **W**=Water.

Species Diversity

To assess the species richness or diversity of a vegetation type, indices were used which attempt to express the diversity of a quadrat or plot by a single number. The best indices combine species richness, totalling of the number of species in a plot and species abundance. One of the most widely used indices is the Shannon Index (**H'**) which accords greater weight to contrasts in rare species and estimated species richness (DeJong 1975). This index was calculated for plots in native forest communities and *Syzygium jambos* invaded plots using Equation 2.10.

$$\mathbf{H'} = -\sum_{i=1}^{s} p_i \, \mathrm{Ln}\, p_i$$

where s = the number of species
p_i = abundance of ith species expressed as a proportion of total cover
Ln = Log base $_n$

Equation 2.10 Shannon Index (H')

Other *Syzygium jambos* forest measurements

Syzygium jambos seedling and sapling density, within each 10x10m plot was calculated by sub-sampling each plot, using five randomly placed 1x1m quadrats, and averaging the counts. In addition every individual adult *Syzygium jambos* in the 10x10m subplot was recorded, and diameter measurements taken at 15cm above ground level. The majority of *Syzygium jambos* adults had been

coppiced in the past. The usual diameter measurement, diameter at breast height (dbh), was difficult to record because of the coppicing, and also it was felt that this would have given an inaccurate estimate of stem volume, because of the large number of small branches at this height (Plate 1). The categories devised for defining seedling, sapling and adult *Syzygium jambos* are outlined in Table 2.4.

Table 2.4. Classification of the *Syzygium jambos* population on Pitcairn Island

Syzygium jambos	**Seedling**	**Sapling**	**Adult**
Height (m)	0.0-0.50m	0.50-1.5m	> 1.5m
Diamter (cm)	<5cm	5-10cm	>10cm

***Plate 1. Syzygium jambos* invaded forest on Pitcairn Island (Plot 6 Upper Mema) showing mature coppiced trees and abundant seedlings.**

The position of each plot and subplot within the invaded *Syzygium jambos* forest relative to remaining native forest communities was measured by tape or estimated from GPS positions where distances were greater than 100m. The altitude at each plot was recorded using an altimeter (Sun-*203M*) and slope was measured using a clinometer (Silva-*ClinoMaster*). Latitude and longitude were recorded using a global positioning system (Garmin-*etrex*) with accuracy range of 3-12m (Table 2.6).

Table 2.6 The location, aspect, altitude and slope of each experimental plot in *Syzygium jambos* dominated forest with four permanent subplots a, b, c & d.

Plot Location	Plot No.	GPS Location	Altitude	Aspect	Subplot Slope a	Subplot Slope b	Subplot Slope c	Subplot Slope d
Flatlands Camp	1	S25°04.079' W130°06.403'	188m	W320°	0°	0°	0°	0°
Pulau	2	S25°03.925' W130°06.264'	190m	N20°	35°	35°	20°	20°
Sires Valley	3	S25°04.164' W130°06.172'	265m	NE70°	20°	20°	20°	30°
Tedside to Sea	4	S25°03.145' W130°06.575'	251m	N0°	40°	30°	20°	30°
Lower Mema	5	S25°03.998' W130°06.871'	220m	N0°	30°	30°	30°	30°
Upper Mema	6	S25°03.010' W130°06.843'	240m	N0°	40°	40°	45°	45°
No Boar	7	S25°04.086' W130°06.559'	260m	W340°	0°	0°	0°	0°
Corner at the Hollow	8	S25°04.049' W130°06.601'	260m	W340°	10°	20°	20°	10°
Devil's Elbow	9	S25°03.965' W130°06.620'	250m	W300°	10°	10°	25°	25°
Up the Flat	10	S25°04.213' W130°06.335'	200m	E150°	15°	15°	15°	15°
White Seed	11	S25°04.106' W130°06.626'	200m	E90°	20°	10°	10°	15°
Up the Cut	12	S25°04.178' W130°06.726'	270m	N20°	15°	15°	20°	20°
Charles Aute	13	S25°04.266' W130°06.491'	200m	NW330°	20°	30°	35°	40°
Graveyard 1	14	S25°04.162 W130°06.523'	140m	W320°	10°	10°	10°	10°
Graveyard 2	15	S25°03.991' W130°06.866'	150m	N0°	5°	5°	20°	15°
Flatlands Mango	16	S25°04.112' W130°03.393'	115m	N50°	0°	0°	0°	0°
Trevor's Canyon	17	S25°04.210' W130°06.330'	148m	E145°	0°	0°	0°	0°
Browns Water	18	S25°04.139' W130°06.273'	60m	W300°	55°	60°	10°	20°
Tonina Valley	19	S25°04.166' W130°06.171'	100m	E90°	30°	30°	40°	40°
Little George	20	S25°04.157' W130°06.240'	170m	W270°	35°	47°	40°	40°
Graveyard 3	21	S25°03.991' W130°06.866'	200m	N0°	35°	35°	40°	40°

2.2.4 Soil sampling and analysis

Soil samples were collected for chemical analysis with a hand trowel; seven cores of soil (4cm wide x 15cm deep) were extracted from random points within each 10x10m plot. Each core sample collected was at least 1.5m apart from the next, and the sample was bulked and mixed in the field. The humus layer was included but the litter layer was excluded. The soil was air-dried on Pitcairn Island and passed through a 5mm sieve before shipping to the Botany department, Trinity College Dublin for detailed chemical analysis. Detailed analysis were performed (see below) on the samples to obtain pH, organic content, total nitrogen, total carbon, carbon:nitrogen ratio and total phosphorus content in *Syzygium jambos* forest soils.

pH

The pH of a solution refers to its hydrogen ion activity and is expressed as $-Log_{10}[H^+]$. pH is based on a Log scale from 0 (very acidic) through 7 (neutral) to 14 (very alkaline). The soil pH of for each site was determined roughly in the field with moist soil (Jenway microprocessor pH meter model 3100) with 1 teaspoon of soil (~10g) to 20ml of distilled water (sealed 10ml strips of distilled water were obtained from the Medical Centre on Pitcairn Island). The pH meter was calibrated in the field using manufacturer supplied buffer capsules. The pH was read using an ion-sensitive electrode on two replicates of each sample.

The pH of air-dried soil (WTW pH330 Meter with Sentix 97/T pH electrode) from each plot and each site was also determined at Trinity College Dublin. The electrode was calibrated using buffers of pH4.0 and pH7.0. (moist soil results in the field, had determined that the soils were acidic in nature). An automatic temperature probe was connected to the pH meter and all samples were taken at laboratory temperature. The following method, modified from Grimshaw (1989) was used: 10g of 2mm sieved soil were weighed and mixed with 20ml of distilled water, the mixture was stirred with a glass rod to form slurry and left to settle for 15 minutes. The pH electrode was placed in the slurry for 3 minutes, during this time the digital display settled and a reading was taken. Between each sample the electrode was washed with distilled water and blotted dry with tissue. There was very little difference between the pH results obtained in the field and in the laboratory. The pH values obtained in the laboratory were used for further analysis.

Organic content by loss on ignition

The organic content of a soil sample can be obtained by burning off the organic matter at temperatures around 550°C, this process is known as loss on ignition. This temperature (550°C) is widely used and is considered the most accurate, as it retains the volatile minerals which may be lost at temperatures above 550°C (Allen 1989).

Two replicates from the bulk soil sample obtained from each plot were oven dried overnight at 100°C in foil cups. Ovenproof crucibles were pre-weighted to four decimal places, and 1g of oven dried soil was added. The weighed crucibles and soil samples were placed in a Thermolyne 6000 furnace and the temperature was gradually increased to 550°C over 1 hour and then left at 550°C for five hours. When the samples were cool enough to handle with tongs, they

were transferred to a desiccator, and allowed to cool to room temperature, and then re-weighed. The percentage loss on ignition, which is equivalent to the organic content lost by each sample on combustion, was calculated in the steps outlined in Equation 2.11:

$$\textbf{Loss on ignition (\%Organic matter)} = \frac{*\text{Weight loss of sample} \times 100}{\text{Oven dry weight of sample g}}$$

(*Weight loss of sample g = (Weight of crucible +oven dry sample g) – (Weight of crucible and ignited sample g)

Equation 2.11 Loss on ignition

Bulk density (Equation 2.12) is the weight of soil per unit volume, which reflects both the porosity and proportion of organic matter in a soil. Bulk density was estimated for the samples from predictive tables which utilize the regression of bulk density on the log of ignition loss, following the method of Jeffrey 1970.

Bulk Density = 1.482-0.6786 x Log **% Loss on Ignition**

Equation 2.12 Bulk Density Calculation

Analysis of total nitrogen, total carbon & organic carbon by elemental analysis (LECO)

Soil samples from plots were pre-treated with sulphurous acid to remove inorganic carbon. Sulphurous acid removes the carbonate carbon without loss of nitrogen. The method used was modified from Verardo *et al.* (1990).

Sulphurous acid digest

A 40ml beaker was weighed and 1.0g of the soil sample was added. The soil sample was wetted by adding 5ml of distilled water. 5ml of sulphurous acid was then added to the sample and placed at 60°C on a hot plate in a fume hood. When a soil sample contains a high carbonate content it will "fizz" on sulphurous acid addition so sulphurous acid has to be added in small 5ml aliquots until no further effervescence occurs. The soil samples from Pitcairn required only one 5ml aliquot of sulphurous acid to remove carbonate carbon. The samples were then evaporated to dryness overnight on a hotplate.

LECO analysis

The sulphurous acid-digested samples were then reweighed and the weight gain recorded (weight normally increases by a small amount). Then 0.2g of the sample was placed in a tin cup and the cup twist sealed and reweighed accurately to four decimal places. Calibration standards of EDTA[2] are prepared by weighing out a smaller amount of EDTA (0.05g) and placing in a tin cup, sealing and

[2] **EDTA**:ethylene diaminetetraacetic acid

reweighing. The tin cup samples were loaded into the LECO elemental analyser wells and gases turned on.

The machine method setting chosen was ORGANIC with preset combustion and oxygen flow rates suitable for soil analysis. The machine counter settings were checked to ensure none were approaching their limits and a gas leak check of the helium, ballast and oxygen systems performed. Five EDTA standards were used to calibrate the machine. The sample weights were entered in the ANALYSE menu and the samples were run along with standard check samples of EDTA (one EDTA standard sample, for every ten samples analysed). The Total Organic Carbon (%TOC and TOC mgL^{-1}) and Total Nitrogen (%TN and TN mgL^{-1}) were corrected using the weight gain from the sulphurous acid treatment in the final result using Equations 2.13 and 2.14.

%TN = %TN (Machine reading) – [%TN (Machine reading) * (% sample weight increase /100)]

Total N (mg N/L soil) = (**%TN*** 10000) * (**Bulk Density**)

Equation 2.13 Total nitrogen

%TOC = % TOC (Machine reading) - [%TOC (Machine reading) * (% sample weight increase /100)]

Total C (mg C/L soil) = (**%TOC***10000) * (**Bulk Density**)

Equation 2.14 Total organic carbon

The carbon:nitrogen ratio (C:N ratio) of the analysed samples was then calculated by dividing TOC by TN (Equation 2.15).

$$\text{C: N Ratio} = \frac{\text{TOC}}{\text{TN}}$$

Equation 2.15 Carbon: Nitrogen ratio

Total phosphorous determination by acid digestion & colorimetry

Total phosphate content of a soil can be considered a crude first approximation of soil fertility. The phosphate supply in soil depends mostly on the phosphate content of the substrate underneath, though some processes such as sorption and microbial activity also affect the supply of PO_4 to plants. This is not true of other minerals such as nitrogen where biological fixation and leaching are more important in the nutrient cycle. Total phosphorus was determined using spectrophotometry after a partial ignition and a nitric acid digestion, an alternative method using microwave digestion was not used as microwave pressures are not considered adequate for soils with low organic content which was the case with Pitcairn soil samples.

Ignition extraction

The ignition/extraction method of Saunders and Williams (1955) was used to determine phosphorous levels in the soil samples. The procedure was carried out as follows: 2g of air-dried sample was weighed and placed in a crucible and partially ignited for 1 hour at 550°C in a Thermolyne 6000 furnace, cooled and transferred to 100ml conical flask.

Nitric Acid digestion

To each flask 50ml of 2M HNO_3 was added and flasks were placed in a water bath at 90°C for 1 hour. Blanks containing only 2M HNO_3 were included as controls. P standards[3] were also prepared and included in the experiment. The flasks were removed and cooled and 40ml of distilled water added. The solution was then filtered using Whatman No.1 Filter papers into 100ml volumetric flasks and made up to the volume of 100ml with distilled water. When the filtered and diluted sample was obtained 0.2ml of the solution was transferred to a clean test tube and the sample further diluted with distilled water to make a final volume of 5ml.

Colorimetry

1ml of mixed reagent[4] (Murphy & Riley 1962; Watanabe & Olsen 1965) was added to the 5ml sample and mixed and left stand for 15 minutes to allow the colour reaction to develop. Absorbance of the sample was read at 882nm on a Shimadzu Europa UV-1601 spectrophotometer. Total P was expressed per volume of soil, using bulk density (Equations 2.16).

$$\textbf{Total phosphate (µg per g)} = \frac{\text{Total P mgL}^{-1} \text{ in digest x volume digest made up to}}{\text{Mass digested in g}}$$

Total phosphate (mgP/L soil) = **Total P** µg per g x **Bulk Density**

Equations 2.16 Total phosphate calculations

A summary of all the environmental variables measured in *Syzygium jambos* plots are presented in Table 2.7.

[3] **P Standards**:1.5mg/L PO_4P; 1mg/L PO_4P; 0.75mg/L PO^4P, 0.5mg/L PO_4P; 0.25mg/L PO_4P & 0.00mg/L PO_4P made up to 100ml with distilled water and 10ml of HNO_3

[4] **Mixed Reagent**: 13.5ml 3.6N H_2SO_4, 25ml antimony stock solution, 25ml Molybdate stock solution and 0.2g abscorbic acid

Table 2.7 A summary of the environmental variables measured, variable type and references used for surveying *Syzygium jambos* forest experimental plots.

Quantitative Environmental Parameter	Measurement	Variable Type	Reference
Location	Global positioning system in the field	Quantitative	
Aspect	Compass in field	Quantitative	
Altitude	Altimeter in field	Quantitative	
Slope	Clinometer in field	Quantitative	
Vegetation cover	Domin scale in field	Quantitative	Kent & Coker 1992
Distance from native forest	Tape measure in field	Quantitative	
Soil pH	Ion electrode in laboratory and field	Quantitative	Grimshaw 1989
Soil organic content	Furnace in Laboratory	Quantitative	Allen 1989
Total organic Carbon	Leco elemental analyser in laboratory	Quantitative	Verardo *et al.* 1990 and Centre for Environment Manual, TCD
Total nitrogen	Leco elemental analyser in laboratory	Quantitative	Verardo *et al.* 1990 and Centre for the Environment Manual, TCD
Total phosphate	Acid digestion & Colorimetry in laboratory	Quantitative	Saunders & Williams 1955; Murphy & Riley 1962; Watanabe & Olsen 1965;Jeffrey 1970
Carbon: Nitrogen Ratio	Leco elemental analyser in laboratory	Quantitative	Centre for the Environment Manual, TCD

2.2.5 Data handling and analysis

Comparisons between and differences in diversity and soil properties (pH and organic matter), in *Syzygium jambos* invaded forest community and the native forest community types as outlined by Kingston and Waldren (2003) were performed. Forty-three, 10x10m subplots were selected, two from each of the 84 experimental subplots in *Syzygium jambos* invaded forest and an additional one drawn at random and analyzed alongside the forty-three, 10x10m plots from within native forest communities using data collected by Kingston & Waldren in 1997 (Kingston & Waldren 2003). Native forest community plots are coded as **Q** throughout and *Syzygium jambos* invaded forest subplots coded with **P**.

In addition, detailed investigations for baseline information on the varying density of *Syzygium jambos,* within *Syzygium jambos* invaded forest were performed using data obtained from all 84 experimental subplots. The additional soil properties of total nitrogen, total organic carbon, total phosphate and the carbon:nitrogen ratio were also calculated out for the 84 experimental subplots within *Syzygium jambos* invaded forest.

Data matrices were compiled for each data set, one for plot/quadrat data and the other for plot/quadrat environmental data using MS EXCEL. Diversity and soil property calculations were performed in MS EXCEL using the equations outlined in Section 2.2.3 and 2.2.4. Graphs were produced in MS EXCEL and standard errors calculated and manually assigned to the graphs.

Canonical Correspondence Analysis (CCA) and Nonmetric multidimensional scaling (NMS) were carried out in PC-ORD Version 4.01 (McCune & Mefford 1999) and ordination diagrams using the results obtained were constructed within PC-ORD Version 4.01 and MS EXCEL.

Correlation and regressions were carried out in DATADESK Version 6.0 (Datadesk 1996) for Windows. Analysis of variance (ANOVA), tests for normality (Cochran's test) and post hoc tests (Student-Newman-Keuls test) were carried out in GMAV5 for Windows (Underwood *et al.* 2004).

2.3 Results

2.3.1 Effect of *Syzygium jambos* forest on native species richness and diversity

Analysis of the vegetation pattern suggested that species richness and diversity of the native flora were affected by *Syzygium jambos* presence. Investigation into both the total species diversity (Figure 2.4) and native species diversity (Figure 2.5) in the two forest types were performed.

The total species diversity was found to differ between native and *Syzygium jambos* forest (Figure 2.4 & Table 2.8). The total mean species diversity in native forest communities (H'=4.89) was more than double the total mean species diversity found in *Syzygium jambos* forest (H'=1.79).

Figure 2.4 Total species diversity (H') in native and *Syzygium jambos* forest

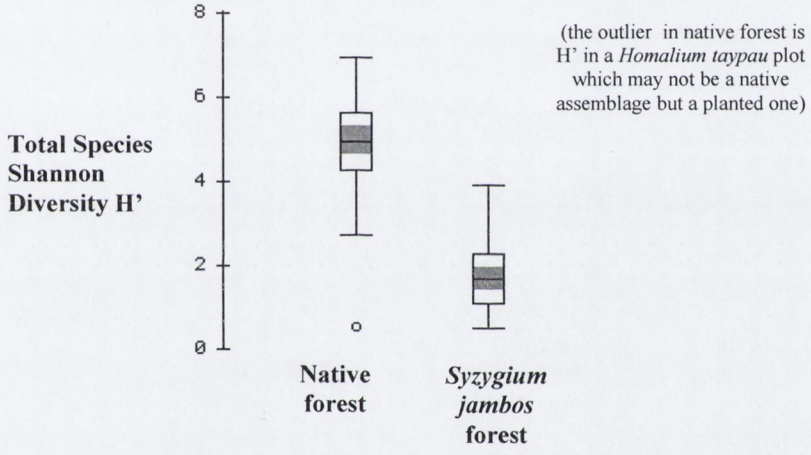


Table 2.8 Mean total species diversity in native and *Syzygium jambos* forest

	Native forest Total H'	*Syzygium jambos* Total H'
Mean	4.89	1.79
St. Dev.	1.30	0.85
St. Err.	0.19	0.13

A Cochran's test of normality was performed on the data were found to be not normally distributed. A log transformation of the data improved normality (C=0.63, not significant [ns]) and the data were subsequently analysed using analysis of variance. Total species diversity was found to be significantly lower in *Syzygium jambos* forest ($F_{[1, 84]}$ 107.73; $P< 0.001$) than in native forest communities (Table 2.9).

Table 2.9 ANOVA table for Total species diversity in *Syzygium jambos* forest and Native forest

Source	*SS*	*DF*	*MS*	*F*	*P*
Forest type	24.88	1	24.87	107.73	0.000 **
Residual	19.39	84	0.23		
Total	44.26	85			

** indicates significance at p<0.001

Native species diversity in the plots chosen was also found to differ between native forest plots and *Syzygium jambos* forest subplots (Figure 2.5 & Table 2.10). The mean native species diversity in native forest communities was (H'= 3.77) more than double the native plant species diversity found within *Syzygium jambos* forest (H'= 1.49) (Table 2.10).

Figure 2.5 Native species diversity (H') in native forest and *Syzygium jambos* forest

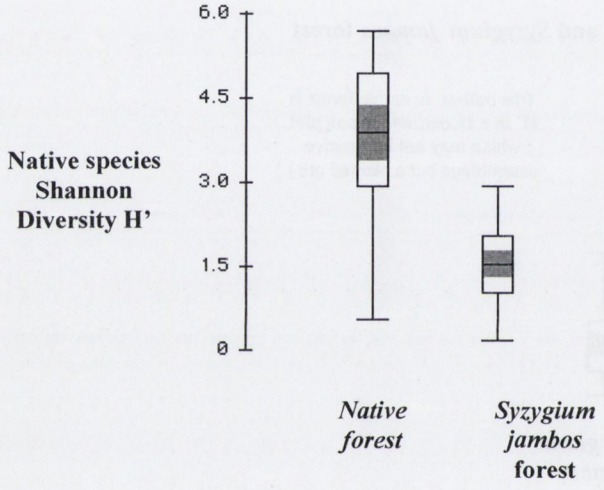


Table 2.10 Comparison of native plant species diversity in native forest and *Syzygium jambos* forest

	Native H'	***Syzygium jambos* H'**
Mean	3.77	1.49
St. Dev.	1.37	0.71
St. Err.	0.21	0.10

The data were log transformed (C=0.59, ns) and analysed using analysis of variance. Diversity in native forest communities was significantly higher (P<0.001) (Table 2.11) with diversity in native plots more than double (Table 2.10) the diversity found in *Syzygium jambos* invaded forest subplots.

Table 2.11 ANOVA table for native species diversity in *Syzygium jambos* forest and Native forest communities

Source	*SS*	*DF*	*MS*	*F*	*P*
Forest type	20.58	1	20.58	67.61	0.000
Residual	25.57	84	0.30		**
Total	46.15	85			

** indicates significance at p<0.001

Within all the *Syzygium jambos* forest sites selected (21 plots; with 84:10x10m subplots) the total species diversity of all 21 plots was calculated (Table 2.13) to investigate if any relationship existed between total species diversity in *Syzygium jambos* plots and proximity (m) to the nearest native forest community.

Table 2.13 Total species diversity within *Syzygium jambos* forest for all 21 sites

	***Syzygium jambos* Total H'**
Mean	1.97
St. Dev.	0.594
St. Err.	0.028

Total species diversity decreased significantly ($P < 0.001$) with distance from native forest (Table 2.14). R^2 values indicate that 60% of species diversity in a plot could be predicted from the distance from native forest. The total species diversity (Ts) could be predicted by the equation Ts = + 2.5273 -0.0025d (Figure 2.6).

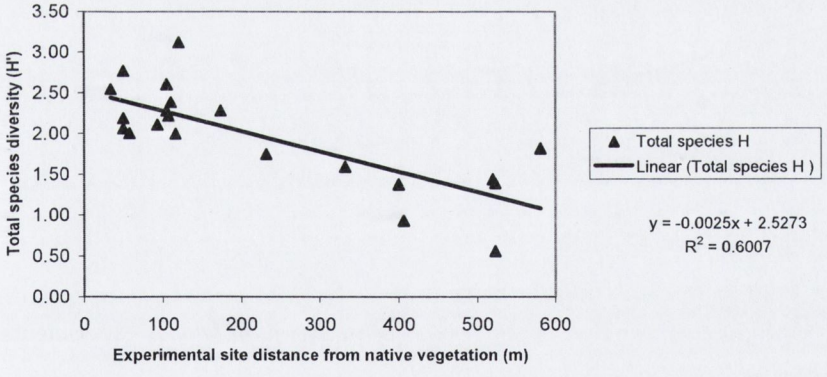


Figure 2.6 The linear relationship between total species diversity in *Syzygium jambos* experimental sites and distance from native forest.

Table 2.14 Linear regression of the total species diversity within *Syzygium jambos* experimental plots and their distance from native forest

Source	*SS*	*DF*	*MS*	*F*	*P*
Regression	4.473	1	4.473	28.7	0.0001**
Residual	2.960	19	0.155		

** indicates significance at p<0.001

The native species diversity was also calculated for the 21 experimental plots within *Syzygium jambos* forest (Table 2.15).

Table 2.15 Native species diversity within *Syzygium jambos* forest for all 21 experimental plots

	Syzygium jambos Native H'
Mean	1.61
St. Dev.	0.470
St. Err.	0.022

Native species diversity also decreased significantly ($P < 0.001$) with distance from native forest (Table 2.16). R^2 values indicate that 60% of native species diversity in a plot could be predicted from the distance from native forest. The native species diversity (Ns) could be predicted by the equation Ns = 2.0456 -0.002d (Figure 2.7) with highest native species diversity found in *Syzygium jambos* plots closet to native forest stands.

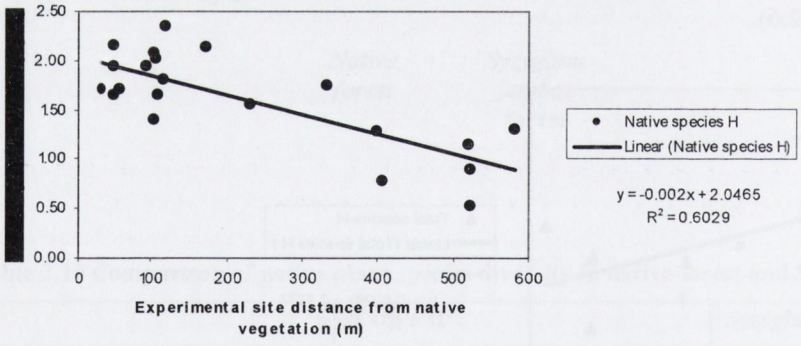


Figure 2.7 The linear relationship between native species diversity in *Syzygium jambos* experimental plots their distance from native forest.

Table 2.16 Linear regression of the native species diversity within *Syzygium jambos* experimental plots and their distance from native forest

Source	*SS*	*DF*	*MS*	*F*	*P*
Regression	2.798	1	2.798	28.9	0.0001**
Residual	1.839	19	0.096		

** indicates significance at $p<0.001$

In the 43 *Syzygium jambos* forest subplots assessed, 35 plant species were found, while in the 43, native forest plots (*Pandanus tectorius* woodland, *Meterosideros collina* woodland & *Homalium taypau woodland*) described by Kingston & Waldren (2003), 73 plant species were found.

Of the 35 species found in *Syzygium jambos* forest subplots, 34.2% were exotic weedy species, 5.7% exotic Polynesian introductions and 2.8% exotic cultivated fruit species. The total non-native species percentage was 42.7% (Table 2.17). In native forest community quadrats, 24.6% of the species found were exotic weedy species, 6.8% exotic Polynesian introductions and 8.2% exotic species cultivated for fruits, giving a total non-native species percentage of 39.6% (Table 2.18).

Table 2.17 Species found in the forty-three *Syzygium jambos* subplots used in the analysis

Species found in the 43 *Syzygium jambos* subplots

Angiopteris chauliodonta
Argusia argentea[a]
Canna indica[a]
Cosos nucifera
Commelina diffusa[a]
Cordyline fruticosa[p]
Cyathea medullaris
Davallia solida
Doodia media
Glochidion pitcairnense
Glochidion sp.
Guettarda speciosa
Hernandia sonora
Hibiscus tiliaceus
Hippeastrum hortorum[a]
Homalium taypau
Lantana camara[a]
Nephrolepis biserrata
Nephrolepis cordifolia
Nephrolepis hirsutula
Oplismenus hirtellus[a]
Oxalis corniculata[a]
Pandanus tectorius
Passiflora maliformis[c]
Phymatosorus commutatus
Phymatosorus scolopendria
Pneumatopteris costata
Polyscias guilfoylei[a]
Psidium guajava[a]
Psilotum nudum
Sorghum sudanense[a]
Synedrella nodiflora[a]
Syzygium jambos[a]
Thespesia populnea[p]
Xylosma suaveolens

Non-native species: a=exotic weedy species; **p**=exotic Polynesian introduction; **c**=exotic cultivated fruit

Table 2.18 Species found in forty-three native forest community plots (Kingston & Waldren 2003) used in the analysis

Species found in the 43 native Forest plots selected	Species found in the 43 native Forest plots selected
Adiantum hispidulum	*Oplismenus hirtellus*[a]
Aleurites moluccana[p]	*Oxalis corniculata*[a]
Amaranthus sp[p]	*Pandanus tectorius*
Angiopteris chauliodonta	*Paspalum conjugatum*[a]
Arachniodes aristata	*Passiflora maliformis*[c]
Asplenium nidus	*Peperomia blanda*
Asplenium shuttleworthianum	*Peperomia pitcairnensis*
Caesalpinia sp.[a]	*Peperomia rapensis*
Canna indica[a]	*Phymatosorus commutatus*
Celtis pacifica	*Phymatosorus powellii*
Cerastium fontanum ssp. *vulgare*[a]	*Phymatosorus scolopendria*
Christella parasitica	*Pisonia umbellifera*
Citrus aurantium[c]	*Pneumatopteris costata*
Cocos nucifera	*Polyscias quilfoylei*
Coffea arabica[c]	*Psidium guajava*[c]
Commelina diffusa[a]	*Psilotum nudum*
Conyza bonariensis[a]	*Psydrax odorata*
Cordyline fruticosa[p]	*Pyrrosia serpens*
Ctenitis cumingii	*Sonchus oleraceus*[a]
Cyathea medullaris	*Sorghum sudanense*[a]
Cyclophyllum barbatum	*Synedrella nodiflora*[a]
Davallia solida	*Syzygium jambos*
Dicranopteris linearis[a]	*Taeniophyllum fasciola*
Diplazium harpeodes	*Thespesia populnea*[p]
Doodia media	*Trichomanes enlicherianum*
Eugenia reinwardtiana[a]	*Trichomanes sp. nov*
Ficus prolixa[p]	*Verbena sp.*[a]
Glochidion comitum	*Vittaria elongata*
Glochidion pitcairnense	
Grevillea sp.[a]	
Hernandia sonora	
Hibiscus tiliaceus	
Homalium taypau	
Jasminum didymum	
Kyllinga sp.[a]	
Lantana camara[a]	
Leguminous tree[a]	
Mangifera indica[c]	
Metrosideros collina	
Morinda citrifolia[p]	
Morinda myrtifolia	
Musa sp.[c]	
Nephrolepis biserrata	
Nephrolepis cordifolia	
Nephrolepis hirsutula	

Non-native species: a=exotic weedy species; **p**=exotic Polynesian introduction; **c**= exotic cultivated fruit

To fully understand the difference in vegetation between the native forest communities (43;10x10m plots) and *Syzygium jambos* forest (84;10x10m subplots) an NMS ordination was conducted using DOMIN species cover values for each plot and the distance measure used was Sorenson (Bray-Curtis).

The final stress for a 2-dimensional solution was 16.7 with a final instability of 0.000010 and 400 iterations. A Monto Carlo test demonstrated that stress (Legendre & Legendre 1998) in the preliminary runs was significantly (P=0.0196) lower than would be expected by chance (Table 2.19). The best 2- dimensional solution from the runs was used as the starting position for the final ordination (Figure 2.7).

Table 2.19 Stress and "p"values for NMS of 6 dimensions for the dataset of DOMIN species values for the 43 plots in Native forest communities and 84 subplots in *Syzygium jambos* forest plots on Pitcairn Island.

	Stress in real data (40 runs)			Stress in randomized data Monto Carlo test, 50 runs			
Dimension	**Min**	**Mean**	**Max**	**Min**	**Mean**	**Max**	**p**
1	26.856	42.639	45.067	39.309	44.860	57.323	0.0196
2	16.744	17.807	18.699	26.172	27.845	47.383	0.0196
3	12.376	13.433	14.543	19.704	22.616	39.143	0.0196
4	11.396	11.943	13.047	15.799	20.108	41.387	0.0196
5	10.664	11.293	12.420	13.700	17.939	33.790	0.0196
6	10.217	10.860	11.543	11.911	16.470	33.439	0.0196

"p" = proportion of randomized runs with stress < or = observed stress i.e., "p" = (1 + no. permutations <= observed)/(1 + no. permutations)

Dimension 2 of the NMS ordination separated native forest communities and *Syzygium jambos* invaded forest. With *Syzygium jambos* forest plots all clustered together on the NMS ordination with mostly positive scores on Dimension 2 and negative scores on Dimension 1 and clustered around zero (Figure 2.7).

Subplot 18B at Brown's Water, one of the *Syzygium jambos* plots, separated from the main cluster of *Syzygium jambos* plots as it contained weedy floral elements such as *Sonchus oleraceus*, *Synedrella nodiflora* and *Canna indica* and some native species such as *Pandanus tectorius*, *Davallia solida* and *Nephrolepis hirsutula.* This plot aligned closest to Kingston & Waldren's Quadrat 4 from *Meterosideros collina* forest, which contained many of the same native species and a small percentage cover of *Syzygium jambos*.

The *Syzygium jambos* plot 5B at Lower Mema also segregated from the main cluster, being close to *Homalium taypau* forest and containing the native species *Nephrolepis hirsutula*, *Nephrolepis biserrata*, *Glochidion sp.*, *Psilotum nudum*, *Pandanus tectorius* and the exotic species *Psidium guajava* which may account for its separation from the main *Syzygium jambos* group. Subplot 1D in *Syzygium jambos* forest also separated from the main cluster. It contained *Guettarda speciosa*, a native tree which is very rare on the Island.

The native forest communities were positively correlated with Dimension 1, and each of the different native forest communities blurred into each other with the *Pandanus tectorius* forest community the least distinct, seen to be integrating with both *Homalium taypau* and *Meterosideros collina* communities (Figure 2.8).

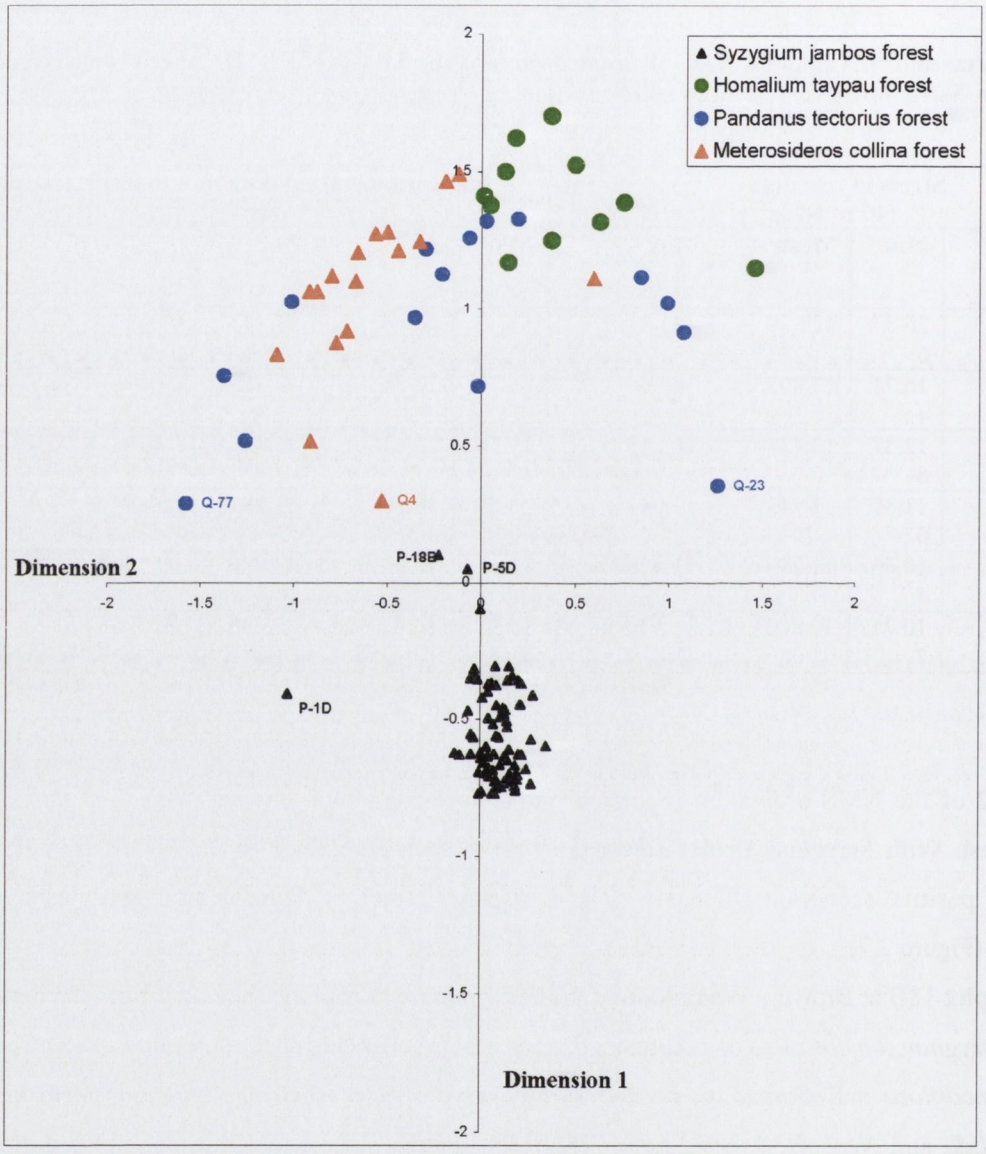


Figure 2. 8 NMS ordination of *Syzygium jambos* forest subplots (P) and Native community forest plots (Q) (Kingston & Waldren 2003) with Dimension 1 plotted against Dimension 2. Labelled plots are discussed in the text.

2.3.3. *Syzygium jambos* density

Syzygium jambos varied in density throughout the plots chosen (Figure 2.9). The plot with the highest density of adult *Syzygium jambos* was plot 15 (Graveyard 2) with 246 adults, the plot had been burnt and cleared previously and the adults were young with the majority with diameters <40cm.

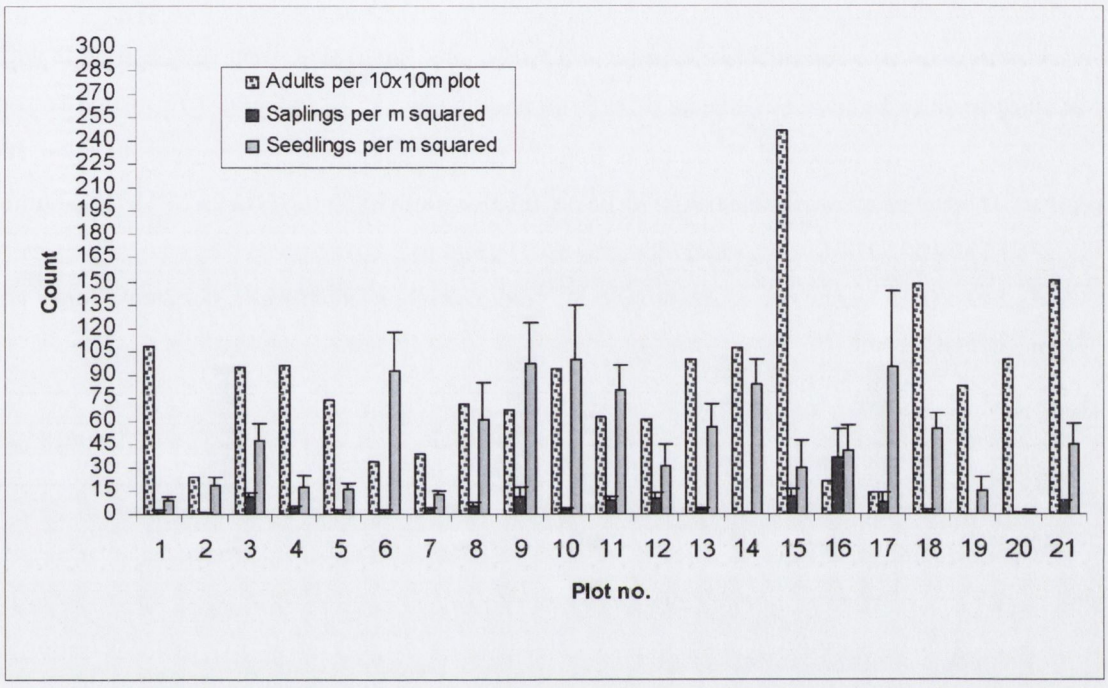


Figure 2.9 Seedling, sapling and adult densities of *Syzygium jambos* at the 21 plots chosen for control treatment.

The highest sapling density was found at plot 16 (Flatlands Mango) with an average of 39.90(± 38) saplings m^{-2}. This plot had been cleared previously and there was evidence of former garden cultivation with *Hippeastrum* x *hortorum* spread throughout the site. The highest seedling density was found at plot 10 (Up the Flat) with an average of 100.5 (± 69) seedlings m^{-2}. The density of *Syzygium jambos* varied greatly within and among plots.

Attempts to explain the variation in seedling, sapling and adult densities across the *Syzygium jambos* forest is difficult as due to the range of variables that could influence the density. Chi^2 tests of seedling density (variance 45.9; $d.f_{[83]}$; $\mathbf{X^2}$ = 389.3; $P \leq 0.0001$), sapling density (variance 11.6; $d.f._{[83]}$; $\mathbf{X^2}$ = 969.6; $P \leq 0.0001$) and adult density (variance 2.0; $d.f._{[83]}$; $\mathbf{X^2}$ = 171.7; $P \leq 0.0001$) revealed that *Syzygium jambos* densities differed in all plots. The sample statistics for the densities are presented in Table 2.20.

Table 2.20 Seedling, sapling and adult density/m^2 in *Syzygium jambos* forest

Density m^{-2}	Mean	Standard Deviation	Standard Error
Seedling	47.7	45.9	5.0
Sapling	6.1	11.7	1.3
Adult	2.3	2.0	0.2

Plots with a large number of adults *Syzygium jambos* had small stem diameters and plots with few adults generally had large diameters. Linear regression gave an R^2 value of 0.76, which 76% of the variation in the in the number of adults (log) in plots is accounted for by stem diameter (Figure 2.11). There was a significant negative linear relationship ($P \leq 0.0001$,Table 2.21). The number of adults in plots = -0.0051x log stem diameter +0.76 (Figure 2.10).

Table 2.21 Linear regression of the number of adult Syzygium jambos in experimental plots and the log of stem diameter.

Source	*SS*	*DF*	*MS*	*F*	*P*
Regression	443312	1	443312	47.1	0.0001**
Residual	141114	19	9407.46		

** indicates significance at $p<0.001$

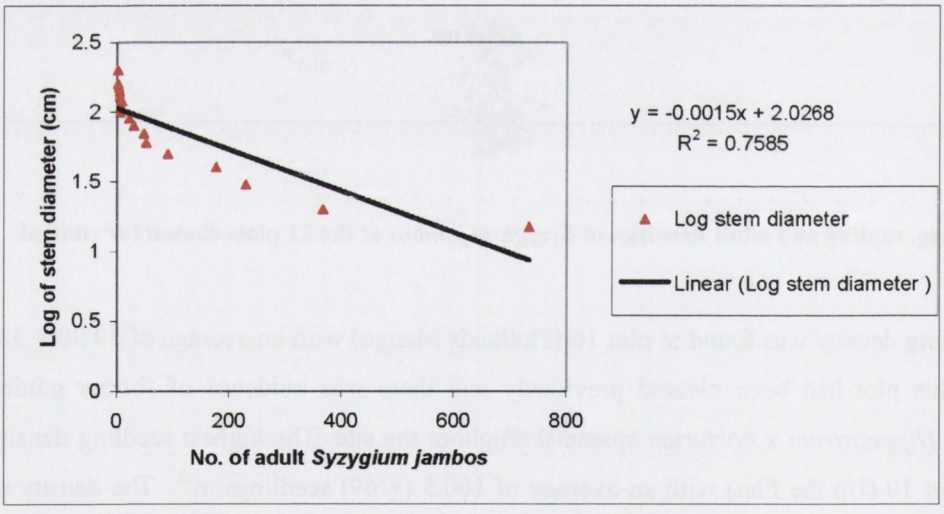


Figure 2.10 The linear relationship between the number of *Syzygium jambos* adults found in experimental plots and the recorded stem diameter.

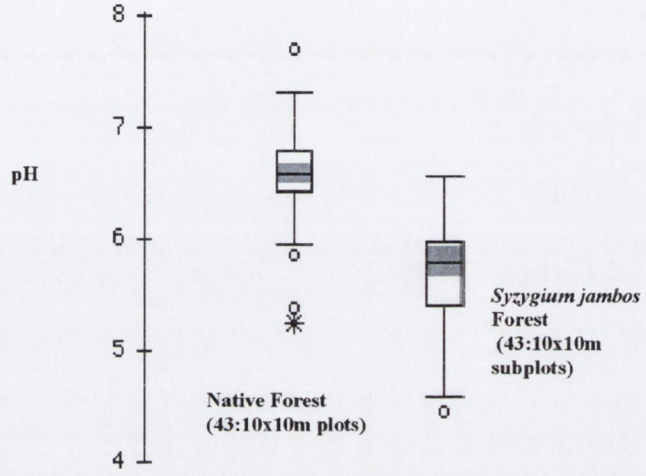
2.3.4 Effect of *Syzygium jambos* forest on soil properties

pH

Results of analysis of the soil pH suggested soils of *Syzygium jambos* forest were different to the native forest (Figure 2.11). Forty-three 10x10m plots were selected two from each of the 84 experimental plots and an additional one drawn at random and analyzed alongside the forty-three

10x10m plots from sites with native forest types; *Homalium taypau* forest, *Pandanus tectorius* forest & *Meterosideros collina* forest identified by Kingston & Waldren (2003).

Figure 2.11 pH content of soils from native forest quadrats and *Syzygium jambos* forest plots



The mean pH values of soils of the plots analysed in both forest types are presented in Table 2.22. The pH of soils in *Syzygium jambos* forest was in general found to be slightly but significantly ($p<0.001$, Table 2.23) lower than the pH of soils in native forest types.

Table 2.22 Mean pH of soil in native and Syzygium jambos forest

	Native forest pH	***Syzygium jambos* forest pH**
Mean	6.60	5.68
St. Dev.	0.44	0.52
St. Err.	0.01	0.01

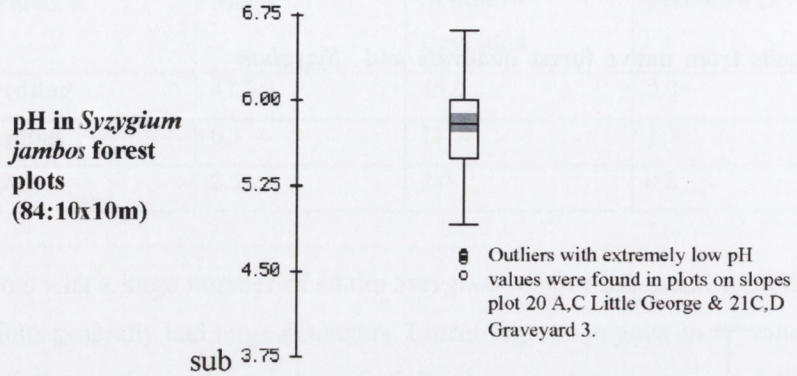
Table 2.23 ANOVA table for pH in *Syzygium jambos* forest and Native forest

Source	*SS*	*DF*	*MS*	*F*	*P*
Forest type	18.22	1	18.22	78.05	0.000**
Residual	19.61	84	0.2335		
Total	37.84	85			

** indicates significance at $p<0.001$

Within all *Syzygium jambos* plots (84 subplots) the pH ranged from a low of 4.64 at plot 21 to a maximum of 6.63 at plot 19 (Figure 2.12), the mean pH was 5.70.

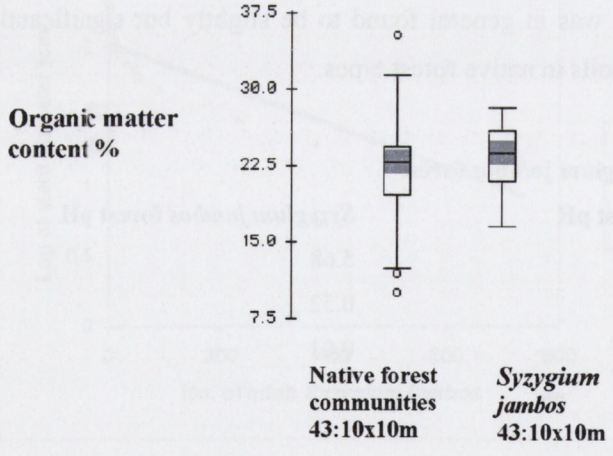
Figure 2.12 pH from all 84 subplots in *Syzygium jambos* forest



Organic matter

Analysis of the organic matter content of soils from 43 plots in native forest (Kingston 2001) and the 43 selected subplots from *Syzygium jambos* forest was carried out. The percentage organic matter was found to be marginally higher in *Syzygium jambos* forest when compared to the native forest communities described by Kingston & Waldren (2003) though there was a large variation in the percentage organic matter found in native forest communities (Figure 2.13).

Figure 2.13 Organic matter content %



The percentage organic matter found in *Syzygium jambos* forest was significantly higher than that found in native forest (Table 2.24) ($t_{[69]}$ =-1.31; p=0.2619).

Table 2.24 Organic matter (%) in native and *Syzygium jambos* forest

	Native forest %OM	*Syzygium jambos* forest %OM
Mean	22.32	23.38
St. Dev.	5.24	3.20
St. Err.	0.12	0.07

Within *Syzygium jambos* forest at 84 subplots the percentage organic matter ranged from a minimum of 14.79 % at subplot 21 B Graveyard 3 to a maximum of 34.28 at plot8; Corner at the Hollow subplot C. In analysis of variance there was asignificant difference between the content of organic matter in Native and *Syzygium jambos* forest.

2.3.5. Other soil properties in *Syzygium jambos* forest

Other soil properties were only measured from the 84 subplots within *Syzygium jambos* forest. The variables measured were Total nitrogen (%TN and $mgNL^{-1}$), Total organic carbon (%TOC & $mgCL^{-1}$) the carbon:nitrogen ratio and the total phosphorus (mg PL^{-1} soil) (Figure 2.16). The summary statistics for these properties are presented in Table 2.25.

Table 2.25 Soil nutrients within *Syzygium jambos* forest

Subplots n=84	Total nitrogen ($mgNL^{-1}$)	Total organic carbon ($mgCL^{-1}$)	Carbon: Nitrogen ratio (C:N)	Total Phosphate (mg PL^{-1} soil)
Mean	2499.03	27699.96	11.90	703.45
St. Dev	787.50	8681.425	4.66	288.28
St. Err	85.92	947.2211	0.52	32.03

The subplot with the highest nitrogen was at Flatlands site 1: subplot 1A (4235.78 $mgNL^{-1}$) and the lowest was at plot 4 Tedside to the Sea: subplot 4A (913.57 $mgNL^{-1}$). The minimum value of Organic Carbon was found at plot 2 Pulau: subplot 2A (9315.19$mgCL^{-1}$) an extremely high value of percentage total organic carbon was found at plot 15 Graveyard 2: subplot 15B (47,004.66$mgCL^{-1}$) this plot has been burned previously, probably in the last fifteen years. The carbon: nitrogen ratio varied from 4.56 at plot 5 Lower Mema: subplot 5A to a high of 20.65 at plot 20 Little George: subplot 20D. Two outlying values of 23.11 and 32.817 were recorded for plot 4: Tedside to the Sea; subplots 4A & 4B. In general the total phosphate levels recorded in *Syzygium jambos* forest were high. The highest value recorded was from plot 16 Flatlands Mango; subplot 16A (1249.0mg PL^{-1}) and lowest values (128.67mgPL^{-1}) from plot 20 Little George; subplot 20A (Figure 2.14).

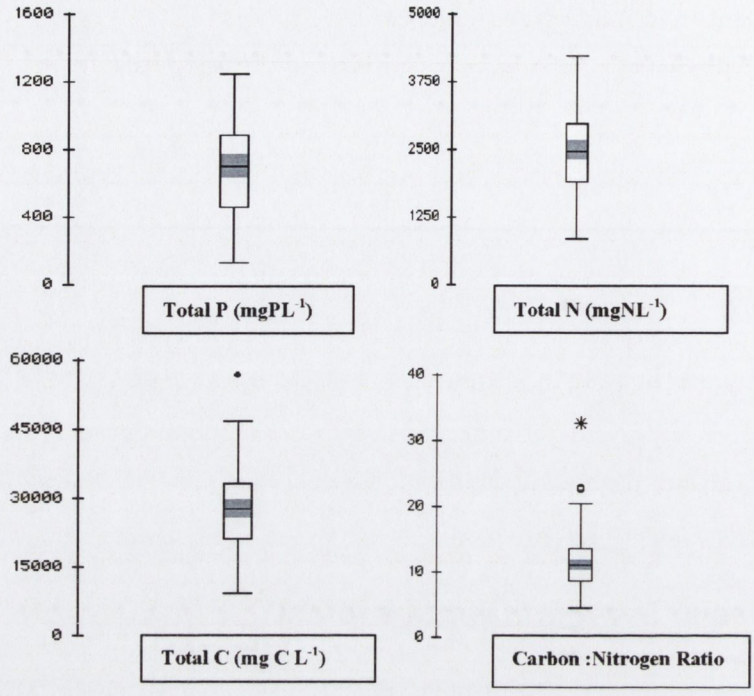


Figure 2.14. Range of values for total nitrogen (mgNL $^{-1}$ soil), total organic carbon (mgCL $^{-1}$ soil), total P (mgPL $^{-1}$ soil) and the carbon: nitrogen ratio (C: N) in *Syzygium jambos* forest (84:10x10m subplots)

The percentage of total nitrogen in soils in the 84 experimental subplots significantly linearly increased with distance from native forest communities (Figure 2.17) the pearson product moment correlation value was 0.42. Linear regression gave an R^2 value of 0.21, which demonstrated that 21 % of the variation in percentage total nitrogen content within soils in *Syzygium jambos* forest could be accounted for by calculating distance from the native forest .The significant linear relationship ($P \leq 0.0001$, Table 2.26) between total nitrogen percentage and slope is; percentage total nitrogen in *Syzygium jambos* forest = -0.0004 x Slope + 0.3711(Figure 2.15).

Table 2.26 Linear regression of percentage total nitrogen and slope within *Syzygium jambos* forest experimental subplots

Source	*SS*	*DF*	*MS*	*F*	*P*
Regression	0.433	1	0.433	21.6	0.0001**
Residual	1.644	82	0.020		

** indicates significance at $p<0.001$

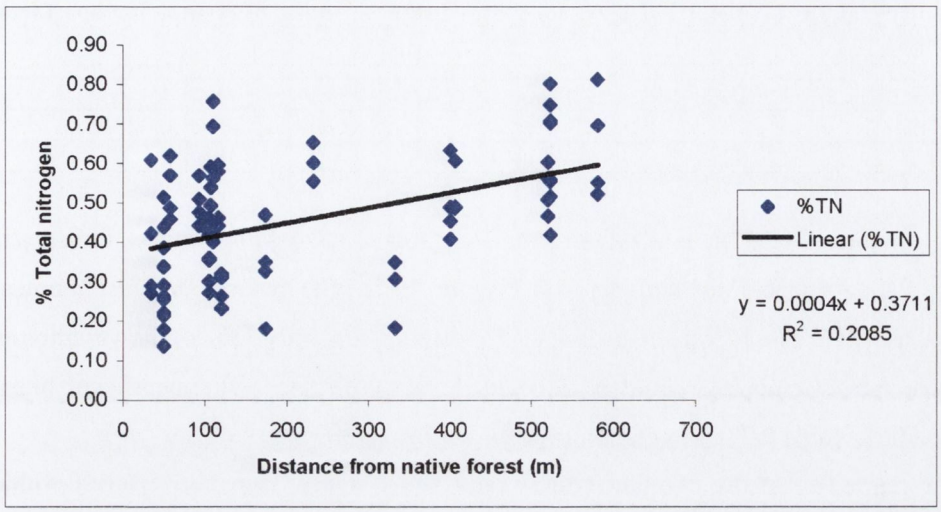


Figure 2.15 Total nitrogen (%) in plots was found to increase significantly with distance from native forest

Total phosphate was found to significantly increase linearly with distance from native forest. The pearson product moment correlation value was 0.53. Linear regression gave an R^2 value of 0.28, which indicated that 28 % of the variation in total phosphate content within soils in *Syzygium jambos* forest could be accounted for by distance to the native forest community. The significant linear relationship ($P \leq 0.0001$, Table 2.27) between total phosphate and distance to native forest = 0.8186 x Slope + 520.66 (Figure 2.16).

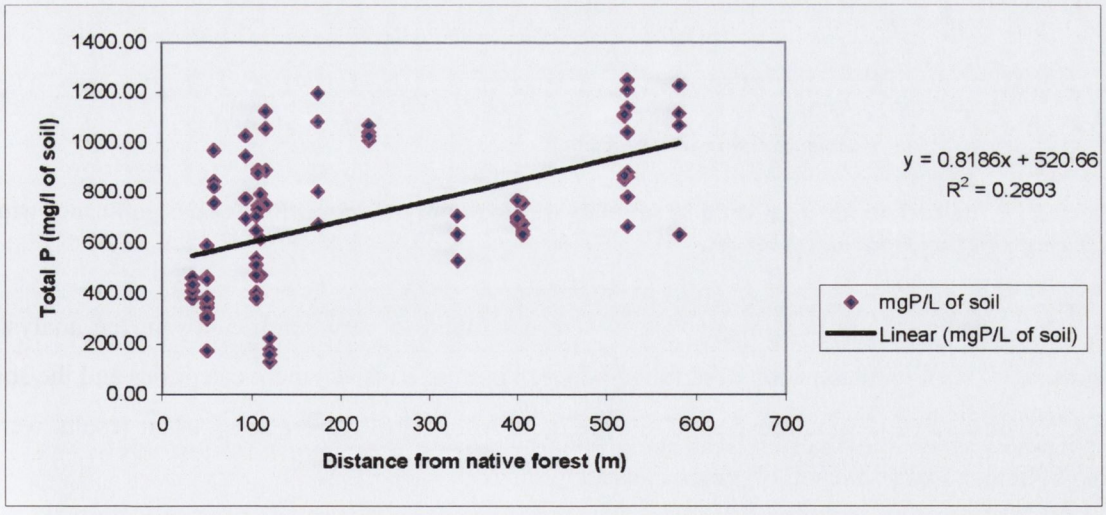


Figure 2.16 Total P (mg/L soil) in plots was found to increase with linear significance with increasing distance from native forest.

Table 2.27 Linear regression of Total P (mg/L soil) and distance within Syzygium jambos forest experimental subplots

Source	*SS*	*DF*	*MS*	*F*	*P*
Regression	1.933^{e6}	1	1.933^{e6}	31.9	0.0001**
Residual	4.96^{e6}	82	60549.9		

The carbon:nitrogen ratio found in the experimental subplots in *Syzygium jambos* forest was decrease linearly with distance from native forest (Figure 2.17). The pearson product moment correlation value was -0.36. The R^2 value was low 0.13 indicating that only 13% of the variation in the carbon:nitrogen ratio in subplots could be accounted for by distance. The significant linear relationship ($P \leq 0.0001$, Table 2.28) was carbon:nitrogen ratio = -0.009 slope + 13.9

Table 2.28 Linear regression of the carbon:nitrogen ratio and distance from native forest within *Syzygium jambos* forest experimental sites

Source	*SS*	*DF*	*MS*	*F*	*P*
Regression	233.42	1	233.42	12.2	0.0008*
Residual	1569.76	82	19.14		

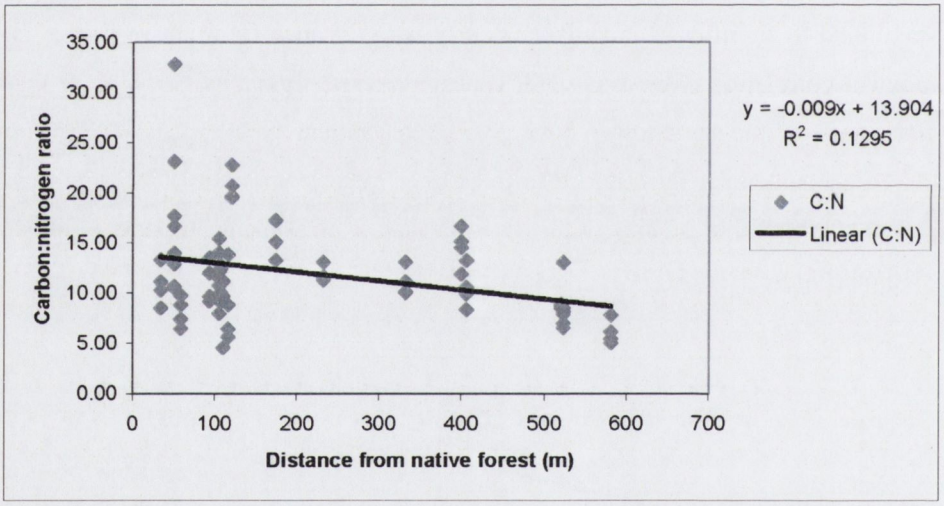


Figure 2.17 The carbon:nitrogen ratio in subplots was found to decrease with linear significance with increasing distance from native forest.

The total organic carbon percentage values were not found to vary significantly in the analysis performed. Also no relationships were found between past land management categories and the soil properties measured despite the fact that some plots with high total organic carbon results were thought likely to be as a result of previous burning.

2.4. Discussion

2.4.1 *Syzygium jambos* and species diversity

Syzygium jambos forest cover was found to affect both the total species diversity and more importantly the native species diversity on Pitcairn Island. The total species diversity in *Syzygium jambos* plots on Pitcairn Island was extremely low when compared with native forest plots. The native species diversity was also extremely low in *Syzygium jambos* forest when compared to native forest plots.

This difference in total and native species diversity between the forest types was significant ($P<0.001$). The total and native plant diversity in *Syzygium jambos* forest was also found to decrease significantly ($P<0.001$) with distance from native forest, suggesting *Syzygium jambos* is affecting the species diversity and only stands in the vicinity of native forest still hold some of the native species.

The species richness of forty-three *Syzygium jambos* forest plots was 35 species of which 63.1% were native plant species. The species richness of forty-three native forest plots was 73 species of which 71% were native species. These results highlight the negative impact of *Syzygium jambos* on Pitcairn Island biodiversity as noted by Waldren (1995), Kingston *et al.* (2004) and Kingston & Waldren (2005). These results also show similarities with *Syzygium jambos* in other invaded regions with similar patterns observed by Brown *et al.* (2006) who also found a significant difference in species number and species richness in *Syzygium jambos* invaded woodlands and primary forest in Puerto Rico.

2.4.2 *Syzygium jambos* and density

The density of *Syzygium jambos* in invaded forest on Pitcairn Island was high. Seedling density was $47.7m^{-2}$, sapling density was $6.1m^{-2}$ and adult density was $2.3m^{-2}$. These values are extremely high in comparison with similar studies in invaded *Syzygium jambos* forest in Puerto Rico (Brown *et al.* 2006) where density ranged from 0.5-0.7 stems m^2. Available comparisons with other shrubby invasive species such as *Rhododendron ponticum* (9.9 seedling m^{-2}) (Erfmeier & Bruelheide 2004) and *Lonicera maackii* (2.13 plants m^{-2}) (Hartman & McCarthy 2004) also demonstrate that *Syzygium jambos* as exists in extremely high densities on Pitcairn Island. It has been generalized that invasive species are highly productive and exhibit greater pressure on invaded communities than in their native habitats (Crawley 1987, Blossey & Nötzold 1995, Erfmeier & Bruelheide 2004). *Syzygium jambos* appears to be much more productive than native tree species on Pitcairn Island as no other tree species on the island appear in such densities (Kingston 2001). A study is needed to compare *Syzygium jambos* density its native habitat and some more habitats where is found to be invading to reveal whether these high density stands are a particular attribute of *Syzygium jambos* on Pitcairn Island.

Syzygium jambos seedling, sapling and adult densities differed within sites in *Syzygium jambos* forest. These densities varied probably because of the historical land use management of the sites in question though this could only be elucidated from observations of sites which had previous being burnt or gardened (Pitcairn islanders *Pers.comm.*). However, no significant relationship was found between site management category and *Syzygium jambos* density. These results are contrary to those of Brown *et al.* (2006) who found that land use history was an important factor which influenced *Syzygium jambos* densities in Puerto Rico.

2.4.3 *Syzygium jambos* forest and soil properties

The soils in native forest and *Syzygium jambos* forest were found to differ significantly in pH and organic matter content. The pH was significantly lower in *Syzygium jambos* forest and organic matter content was also significantly lower in *Syzygium jambos* forest. These differences can perhaps be accounted for by increased run off and loss of cationic minerals in *Syzygium jambos* forest with less litter input from *Syzygium jambos* forest vegetation, less microbial activity in the soil as well as increased erosion.

Twyford (1958), in his analysis of the soils on Pitcairn Island, did indeed note that some differences in soils on Pitcairn were due to plant cover, such as loss of organic matter in areas where gardens have been in existence for a long time. He also noted an increase in soil depth under *Lantana camara* and *Syzygium jambos* and definite evidence of leaching in flatland soils where *Syzygium jambos* dominates (Twyford 1958). Twyford took samples from an area in Flatland which he describes as "sparsely used for garden and fruit tree and much of it covered in Roseapple (*Syzygium jambos*) thicket or secondary bush containing much guava (*Psidium guajava*)" in 1958, his results gave a value pH of 5.65 for the area in Flatlands which is similar to the results obtained in this study. The variances in pH and organic matter in soils in the different native forest types do indeed suggest the influence of vegetation on soil conditions (Kingston 2001).

The difference in soil organic matter and pH between *Syzygium jambos* and native woodland may possibly be a factor in the observed decreased diversity in *Syzygium jambos* plots, by depressing soil microbial activity a feature of invaded ecosystems highlighted by Kourtev *et al.* (2003). Low pH promotes the availability of Al, Fe and Mn which may result in toxic soil concentrates of the available forms of these elements and in turn influence the vegetation (Muller-Dombois & Loope 1990). Future investigations into the reversibility or otherwise of these soils factors in restored former *Syzygium jambos* forest with vegetation cover change from restoration efforts (Chapter 3) would prove an interesting study.

Within *Syzygium jambos* forest investigations into the Total Nitrogen (%), Total Organic Carbon (%), Carbon: Nitrogen ratio and Phosphorus (total P mg l^{-1}soil) were carried out. The values in general are higher than those set out as the general conditions found in tropical soils (Sanchez *et al.* 1982) demonstrating the high levels of soil fertility still to be found within *Syzygium jambos* forest on Pitcairn. Total nitrogen was found to increase significantly in *Syzygium jambos* forest with

increased distance from native forest. Extremely high levels of Phosphorus (703.45 ±288.28 mgPL^{-1} soil) were found in *Syzygium jambos* forest soils. Values above 500 (mg PL^{-1} soil) are seldom encountered without fertiliser addition. It was not surprising to find abnormally high levels of phosphorus, as high levels of phosphorus in Pitcairn soils were noted in the 1950's. The high phosphate levels prompted Twyford (1958) to investigate Pitcairn soils with the view to finding an exploitable phosphate deposits. The high levels were found to be associated with high amount of phosphate minerals in the underlying rocks but were not deemed of suitable quality or quantity for mining (Twyford 1958). High levels of phosphorus were also recorded in invaded *Syzygium jambos* forest in Puerto Rico (595-959kg/ha) but in their study it remained unclear as to whether the *Syzygium jambos* invasion was a cause or consequence of the observed soil conditions (Brown *et al.* 2006). Further analysis of phosphorus contents of Pitcairn soils is required to determine whether the high phosphorus levels found within *Syzygium jambos* invaded forest on Pitcairn Island are different from phosphorus levels in the other vegetation communities on the island. The carbon:nitrogen ratio which is a general indicator of soil fertility, demonstrated that within *Syzygium jambos* soils decreased in fertility with distance from native forest. The decrease in fertility may possibly indicate high levels of leaching within *Syzygium jambos* forest, this phenomenon was elucidated to by Twyford (1958).

Overall the soil conditions found within *Syzygium jambos* forest may either be as a result of *Syzygium jambos* cover or the remaining conditions relating to previous cultivation efforts. The soil properties investigated within *Syzygium jambos* forest do demonstrate significant differences from native forest (pH) and differences with increasing distance from native forest (Total N, Total P and C:N ratio). Further studies are required to elucidate the "native" state of Pitcairn soils. Twyford (1958) stated in his report that no "natural" soils for study exist on Pitcairn Island as the original forest of the island has disappeared. However, small patches of native *Homalium taypau* and *Meterosideros collina* forest with few non-native species do still remain.

2.5 General conclusions

The invasive species *Syzygium jambos* was found to affect all components of the environment on Pitcairn Island from biodiversity with both total and native species diversity adversely affected by its presence and ecosystem processes such as soil chemistry with both the pH of the soil and organic matter significantly different in invaded forest when compared to native forest. The general soil conditions in *Syzygium jambos* invaded forest when found to be comparable to tropical forest soils, though further investigation into its effects on phosphorous levels and comparisons to the other vegetation communities on the island would be useful. The results here show similarity to results obtained from studies in Puerto Rico in invaded *Syzygium jambos* forest with *Syzygium jambos* adversely affecting species richness and diversity though land use history was an important factor. The diversity and soil conditions found within *Syzygium jambos* invaded forest on Pitcairn Island may be facilitating further invasion.

Upon dealing with invasive species "*shoot first, ask questions later*"
Simberloff (2003)

Chapter 3

Syzygium jambos (L.) Alston control on Pitcairn Island

3.1 Introduction

3.1.1 General approaches to Invasive plant species control

Invasive plant species and weeds are often more competitive than native plants, it is thought that this is often because of the lack of natural controls such as diseases and predators, in the habitats they invade (Keane & Crawley 2003; Lake & Leishman 2004). Once established in an area, the control or eradication of invasive plant species can pose a major challenge (Allendorf & Lundquist 2003).

Eradication is defined as "the removal of every potentially reproducing individual of a species from an area in which reintroduction will not occur" (Myers & Bazeley 2003). Though many programmes initiated to deal with invasive species refer to eradication, these are in most instances control programmes; with control defined as "the reduction of the density of the target species or a reduction in its rate of spread" (Myers & Bazeley 2003). The importance of eradicating or controlling established invasive species which threaten ecosystems, habitats and species is set out in the United Nations Convention on Biological Diversity (CBD) which states that parties to the convention should "prevent the introduction of, control or eradicate alien species"(IUCN 2000).

There are two main approaches to control and eradication of invasive plant species. The first approach is to carry out extensive research on the population biology and the impact of the exotic species in its new environment, before formulating a plan towards control efforts (Donlan *et al.* 2003). The second contrary approach, is that research into and understanding the population biology and the impacts are not necessary, the "shoot first, ask questions later" approach of Simberloff (2003) and the "unaffordable luxury of research which will provide information only for the eulogy" of Coblentz (1990).

In a survey of literature published in the Journal Conservation Biology from 1991 to 2002 using the keywords; exotic, alien and introduced; Donlan *et al.* (2003) identified 86 papers dealing with the impacts and population biology of exotic species, 8 discussing conservation strategies towards the management of them and a mere 6 dealing with the benefits of control or eradication of exotic species. He found no papers dealing with research on eradication techniques. Simberloff (2001) also highlighted the lack of publications on removal efforts and techniques in other international journals. Mack & Lonsdale (2002) cite many instances where the opportunity to eradicate invasive plants were lost due to delayed action, and found very few examples in the literature where early action, aided invasive exotic plant eradication.

While it may not be necessary to understand all aspects of the population biology and impacts of an invasive species, it can be important to understand some, for example, knowledge of the biology can aid the timing of herbicide application, though this may not be essential to the eventual removal of an invasive species. Dixon *et al.* (2002) demonstrated that eradication efforts have to be somewhat informed on the biology of the species, as much of the chemical control efforts to eradicate Buffel grass (*Cenchrus ciliaris*) on Arlie Island, Western Australia were wasted. This was due to the timing of herbicide application, which was either too early and missed many of the seedlings, or too late applied when the plants were senescing.

In a review of the necessary criteria for a successful invasive plant species eradication programme, Myers *et al.* (2000) deemed the only amount of biological information actually needed on an invasive species was that it is, "easy to find and kill, with little or no seed bank". The other six criteria they set out related to sufficient resources and good project management. Clout & Veitch (2002) also discount biological information in outlining conditions for eradication success, mentioning only the need to remove a species faster than it reproduces. They highlighted the importance of other factors such as planning, commitment, preventing re-invasion and support from local people, as standard conditions for success of eradication and control efforts. Cronk & Fuller (2001) outlined nine biological and ecological characteristics, which should be noted to aid the control of invasive species, these are: seed dispersal mechanism, seed ecology, breeding system, rate of growth, ability to re-sprout after cutting, requirements for germination and establishment, environmental factors, susceptibility to pest and disease and comparative ecology, which involves finding out what are the likely species to replace it after control measures.

In a more recent review, Caesar (2005) outlined one single standard for judging success in dealing with invasive plant control, that of: a significant reduction in the density of the target weed, which he termed "biological success". Only then, he feels, is it valid to specify the various elements which have lead to that success. Academics have been criticized for pondering too long on investigations into the traits that make a good "invader" or what ecosystems are most invadable (Ewel *et al.* 1999) rather than gathering information on control efforts (Simberloff 2001).

3.1.2 Control of Invasive plant species on Islands

The paucity of island floras has sparked the transfer of large numbers of exotic species to islands by people (Whistler 1991), to such an extent that many oceanic islands now have many more non-native plant species than equivalent areas of mainland (Lonsdale 1999). Over 50% of the vascular plant species in New Zealand are non-native (Myers & Bazeley 2003). These introduced exotic species have a particularly large impact, because island floras are impoverished and less ecologically resistant.

The features of islands which make them particulary interesting and of immense conservation value also makes them more prone to naturalisation and invasion by exotic species (Mack 1996; Cronk & Fuller 2003). The nature of islands floras, with their paucity of indigenous species, allow niche

space for exotics, and the usually small plant populations with their lack of competitive ability, allow exotic species to flourish (Cronk & Fuller 2003).

While these features make islands more susceptible to the worst effects of invasive species, islands also have advantages over mainland areas when dealing with invasive species. The main advantage is that once eradicated the possibility of re-infestation is decreased on islands, mainly because of the strong dispersal barrier that the surrounding ocean represents, which means eradication or control campaigns can be more successful (Mack & Lonsdale 2002). Also in many instances the smaller area available to exotic species, means that they can be noticed quickly on islands. Although, in some cases, being noticed alone does not lead to a successful eradication effort.

The infamous *Clidemia hirta* in Hawaii was noticed to be escaping from gardens in 1949. The population had expanded to cover less than 100ha in 1952, but the warning signs were ignored and it was described as "uncommon and not dangerous" in 1961 (Smith 1992). Its range in 2001 was estimated at a staggering 40,000ha (Cronk & Fuller 2001). This example serves to highlight the ease with which island invasive species are quick to invade but can also be noticed early. This example highlights the disastrous consequences of delayed action when dealing with invasive species, especially on islands (Mack & Lonsdale 2002).

Eradication of most invasive plant species often meet with little or no success and are considered more difficult than vertebrate eradication, for example, attempts to eradicate 50 different plant species in California met with no success when the infested area exceeded 100ha (Simberloff 2001). The only reported success with large scale invasive plant eradication has occurred on Laysan Island, Central Pacific Ocean. The sandbur, *Cenchrus echinatus,* which covered and dominated 30% of the 85 hectares of Laysan, appeared to be completely eradicated in 2000 (Flint & Rehkemper 2000; Simberloff 2003). Controlling invasive species on islands is often more challenging than on mainland sites. The control effort on Laysan Island involved a huge logistical effort in terms of staffing, supplies and vessel charter to reach Laysan Island as it is five days sail from Honolulu (Flint & Rehkemper 2000). Many invasive species control programmes on islands "ooze machismo" (Simberloff 2001) with the invasive species pitted as a dangerous enemy and control efforts seen as winning a war to the death, for example, the programme to oust red mangrove in Oahu (Hawaii) used 26-tonne amphibious assault vehicles (Simberloff 2001).

3.1.3 The soil seed bank and invasive plant species

The term "seedbank" has been used to describe the reserves of viable seeds present in the soil and on its surface (Roberts 1981). In the past, authors (Major & Pyott 1966) have suggested that the seedbank actually forms part of the flora and determines the plant community, even though the plants themselves are not evident. Others have suggested that in degraded plant communities the soil seed bank holds the "memory" of the original plant community (Bakker *et al.* 1996).

However, not all species form persistent seedbanks. Plant species with large seed (>0.5mm) do not form persistent seedbanks, but are more likely to form transient seedbanks which can persist for short periods only, generally less than one year (Bakker *et al.* 1996). The formation of a transient as opposed to a permanent seedbank is common for dry tropical woody forest species (Ogden 1985; Demel & Granström 1995; Drake 1998) and tropical forest seeds are in many cases short-lived (Garwood 1989; Holl *et al.* 2000). Simberloff (2001) noted that invasive plants with persistent soil seed banks are more difficult to eliminate than those without this feature. It is also thought that alien species tend to form more persistent seed banks while native species form transient seed banks (Drake 1998).

In invasive plant studies, the significance of the soil seed bank is in determining the future vegetation of a site given a disturbance event or after eradication efforts. In Hawaiian forest, the seedbank in *Meterosideros polymorpha* forest was dominated by alien species. This suggested that if *Meterosideros polymorpha* forest was disturbed, alien plants were most likely to increase in abundance (Drake 1998). *Alliaria petiolata* (garlic mustard) eradication efforts in Ohio were hampered by the presence of a persistent seedbank. It was discovered that the population of *Alliaria petiolata* did not substantially decrease and in some instances the population actually increased after eradication efforts which suggested the importance of buried seed in allowing *Alliaria petiolata* to persist despite eradication efforts (Carlton & Gorchov 2004). Some invasive species, however, do not form permanent seedbanks and this characteristic can be used as part of a control strategy, for example; *Celastrus orbiculatus* (oriental bittersweet) in North America (Ellsworth *et al.* 2004). The removal of adult plants of *Celastrus orbiculatus* prior to seed rain resulted in effective control of the species. In South African fynbos vegetation invaded with *Acacia saligna,* the same was true, the seed bank consisted solely of fynbos species, suggesting that control of above ground stands of *Acacia saligna* prior to seed rain was what was required to restore native fynbos vegetation (Holmes & Cowling 1997).

3.1.4 General methods used in Invasive plant species control

There are books outlining control methods for invasive plant species (Cronk & Fuller 2001) and many locally based manuals based on what was learnt locally through trial and error, for example, the Norfolk Island weed control manual (Environment Australia 1997) which outlines recommended treatments for ten weedy invasive species. Motooka *et al.* (2002) cite chemical and physical treatment methods for fifty-three weed species in natural areas of Hawaii.

The three main methods of dealing with invasive plant species are physical control, chemical control, and biological control. They can be used separately or in conjunction, each method has its own advantage and disadvantage. Integrated management using a combination of methods is considered the best by many practitioners (Myers & Bazley 2003).

Physical control

Prior to the development of herbicides, weedy species were removed by mechanical or manual control, by pulling weeds, grubbing with hand tools or in more extreme cases, bulldozing and dragging. The appeal of such methods is that they allow volunteers to get involved in invasive species control (Myers & Bazely 2003).

However, the timing of cutting and pulling is very important, for example when *Cytisus scoparius* was pulled when flowering and seeding in British Columbia, the resulting soil disturbance, seed spread and trampling proved ideal conditions for the germination of even more *Cytisus scoparius* through the seed that was spread, and from dormant seed from the seedbank (Myers & Bazely 2003). Comparisons of control techniques for *Berberis thunbergii* showed that cutting and pulling methods were not as effective as cutting and applying herbicide to stumps. Manual control is considered only feasible when the cover of an invasive species is low (Myers & Bazely 2003).

Burning is a commonly prescribed physical control treatment, but even in fire adapted vegetation types, it often fails to have a positive impact on native species diversity, this was the case with fire treatment of invasive *Pinus* in South Africa (Holmes *et al.* 2000). In some instances fire can promote the cover of an invasive species; this proved to be the situation with the invasive species *Melaleuca quinquenervia* in Florida (Turner *et. al* 1998). Prescribed fire treatments can also alter the nitrogen and carbon dynamics in an ecosystem and promote fire tolerant alien species (Rhoades *et al.* 2002).

Chemical control

Chemical control of invasive species is commonly recommended in conjunction with physical control (Ogden & Rejmánek 2005; Tye *et. al* 2002; Flint & Rehkemper 2002; Environment Australia 1997; Motooka *et al.* 2002). The main advantage of using herbicides to control invasive species is that they are both cost effective and quick acting when compared to manual treatments which are labour intensive and slow.

There are many types of herbicide on the market, and the choice of which one to use depends on the prioritisation of three factors; efficiency, economics and environmental protection (Motooka *et al.* 2002). Herbicides can also be chosen for their selectivity; for example, 2,4-D is useful for herbaceous broadleaved weeds but is ineffective on most woody plants (Motooka *et al.* 2002). Commonly used broad spectrum herbicides are [5]Picloram and Glyphosphate[6]. Both are "non-selective" herbicides, they will kill and injure all plants to which they are applied.

Glyphosphate has been used widely to control and eradicate invasive species, for example *Allairia petiolata* in Ohio (Carlson & Gorchov 2004), *Pueraria phaseoloides* and *Rubus glacus* in the Galápagos Islands (Soria *et al.* 2002), *Cenchrus echinatus* at Laysan Island, Central Pacific

[5] 4-amino-3,5,6-trichloropicolinic acid potassium salt

[6] Isopropylamine salt of Glyphosphate, Water, ethoxylated tallowamine surfactant, organic acids of Glyphosphate & excess Isopropylamine

Ocean (Flint & Rehkemper 2002), *Phalaris arundinacea* in North America (Foster & Wetzel 2005), and *Lonicera maackii* in Ohio (Hartmann & McCarthy 2004).

Glyphosphate (Round-up, Round-up Renew, and Glyphosphate Gold™; Monsanto: 360gm/l (36%) Glyphosphate) is a growth inhibitor in plants. It causes plant mortality by disrupting the synthesis of aromatic amino acids and proteins. Upon application Glyphosphate is transported readily through the phloem and xylem. It is strongly absorbed in soils and readily decomposed by soil micro-organisms; its half–life in soil is 47 days. The oral LD_{50} , that is the lethal dose which kills 50% of a test population of rats, is 4900mg/kg, which is considered almost "non-toxic" (Motooka *et al.* 2002). The advantages of using Glyphosphate are its effectiveness and low toxicity to both the environment and humans. The main disadvantage of Glyphosphate is that rainfall within six hours of application washes it off easily in wet conditions second application is required (Motooka *et al.* 2002). The most prohibitive factor is however, the expense in comparison with other broad spectrum herbicides (Round-up: €110 for 20l which treats 1ha).

Picloram is another broad spectrum herbicide and has been used to treat invasive plants such as *Citharexylum gentryi* in the Galápagos Islands (Soria *et al.* 2002) and *Potentilla recta* in Montana (Lesica & Martin 2003). Picloram (*Tordon Brush Killer* ™ Dow Agro Sciences) is a hormonal herbicide absorbed by both foliage and roots and transported in the xylem and phloem. It is persistent with a half-life of 20-300 days and mobile in soils which are sandy and low in organic matter. In Hawaii a special permit is required from the department of Agriculture for its use and it is recommended as a spot target spray only because of its groundwater contamination potential, it is sensitive to photodecomposition. The oral LD_{50} for rats is 8200mg/kg which is even less toxic than Glyphosate (Motooka *et al.* 2002). The advantages of using Picloram are its effectiveness, low toxicity and cheap price (Tordon: €50 for 1l which treats 3 ha). Its main disadvantage is its potential to cause environmental degradation by contaminating ground water.

The method of herbicide application can vary between foliar methods where the herbicide applied to actively growing leaves, and stem methods where the herbicide is applied to the cut surfaces of stems. With both foliar and stem methods high volume spraying is the most common application method. With stem methods, for example, the trunks are cut close to ground level and the herbicide solution applied on the entire cut surface (Environment Australia 1997).

Additional stem methods include frilling where all bark is cut through to the sapwood and the chemical applied to the sapwood while the sap is still wet (Environment Australia 1997). Other variations of stem methods include notching, where a notch of bark is removed and the chemical applied (Motooka *et al.* 2002), and stem injection, where chemical is injected using a high pressure lance or gun to input the chemical in capsule form into the stem (Hartmann & McCarthy 2004).

Biological control

Biological control is the "holy grail" of invasive plant species control and is fraught with ethical dilemmas and sensational failures, for example, *Opuntia* species and its biological control agent the *Cactoblastus cactorum* moth, in Florida. This moth unfortunately is spreading southwards from

Florida, and now threatens many rare native *Opuntias* in Mexico, the centre of cactus diversity. The same moth species used in Australia, did an excellent job in controlling invasive *Opuntia monocantha* (Myers & Bazely 2003). There are also other reported biocontrol successes in Hawaii with a wide range of invasive plant species, such as, *Lantana camara* (Lantana), *Senna surattensis* (Kolomona) *Passiflora tarminiana* (Banana poka) and *Pennisetum clandestinum* (Kikuyu grass) (Trujillo 2005). Simberloff and Stiling (1996) argue that biocontrol can produce excellent results and rather than abandon biological control they insist that control agents must not be pronounced safe until research supports this conclusion, and advocate greater research efforts into biological control.

The main message from all the methods described above available for invasive plant species control, is evaluate the control methods chosen by field trials initially. A good example of why a pilot study is required can be demonstrated with invasive *Alliaria petiolata.* Regardless of whether it was pulled, cut or sprayed with herbicide, the density declined by the same amount in all treatments including the untreated control plots. This was explained by the natural population dynamics of the species, in which density fluctuates naturally alternating in high and low density years (Myers & Bazely 2003).

3.1.5 Cost of Invasive plant species control

The monetary figures that surround invasive plant control and estimated costs are staggering. The cost of control for the invasive species in the United States is estimated at $120 billion annually and introduced weeds are said to account for $24 billion of this in annual crop losses (Pimental *et al.* 2005). The extent of habitat that invasive plants occupy in the U.S is also shocking, 100 million acres and spreading at a rate of 3 million acres a year, though the total area of the USA is 2.3billion acres the spread of invasive plant species is still significant (Myers & Bazeley 2003).

Given the large amounts of money being spent on invasive species control an interesting amalgamation of two disciplines, ecology and economics has been expanding. Economic analysis has been performed on control efforts for whole countries (Pimentel *et al.* 2005) and territories (Rayment & White 2007), to a general analysis of control projects (Born *et al.* 2005), to costing control efforts for an individual species (Dehnen-Schmutz *et al.* 2004). Research is becoming more and more focused on getting "bang for buck" when it comes to invasive species control (Buhle *et al.* 2005).

The cost of invasive species control though can be hugely variable which is more dependent on the country dealing with the invasion and the nature of the invasive species itself, so it can be difficult to generalize about costs. Some examples of costs per hectare for control of invasive species are: *Rhododendron ponticum* control in the UK; £526 per hectare in 2001 (Dehnen-Schmutz *et al.* 2003) and non-native species removal from South African Fynbos from US$140-830 per hectare (Rayment & White 2007). The highest reported cost of invasive species control comes from California, with costs per hectare ranging from US$600 to US$75,000 per

hectare, dependant on the densities and concentrations of the invasive species being dealt with (Rejmánek *et al.* 2000).

Not surprisingly, because of their isolation, the control of invasive plant species is very expensive on islands. For example, the Laysan Island sandbur eradication programme, costs $2600 per hectare per annum (Flint & Rehkemper 2000). On the island of Mauritius the estimated cost for invasive species control is in the region of US$3,000 per hectare (Rayment & White 2007). Mauritius is so badly invaded that only a small part of the natural vegetation can be preserved, and because it is so precious, intensive hand weeding rather than a large scale chemical method is required, also staff with more specialized skills are needed they have to be able to identify the invasive weeds from the native plants (Cronk & Fuller 2001).

3.1.6 Aims of this study

The main aims of the investigations performed in this chapter were to test the efficiency of the commonly used physical and chemical treatment methods on the invasive species *Syzygium jambos* as a pilot study for its control, from which further control strategies can be recommended. The specific aims are:

1. To test the efficiency of chemicals and treatment methods for the control of *Syzygium jambos.*
2. To investigate the composition of the seedbank in *Syzygium jambos* dominated vegetation.
3. To access the cost of treatments on *Syzygium jambos* and make recommendations for its control on Pitcairn Island.

3.2 Methods

3.2.1 *Syzygium jambos* control: plot selection

The twenty-one (20x20m) plots, with eighty-four (10x10m) subplots nested within, which were selected for investigations into vegetation, diversity and soil nutrient composition in Chapter 2, were chemically and physically treated over a two year period during 2003 and 2004 after diversity, soil analysis (Chapter 2) and seedbank assessment (Chapter 3: section 3.2.3) (Figure 3.0)

Figure 3.0. Plot design: 20x20m plot divided into four subplots (divided with red lines). FI = frill treatment (see below) and CP=cut treatment (see below). NS=native seed sown (see Chapter 4)

3.2.2 *Syzygium jambos* control: experimental treatments applied

Two experimental control treatments for adult *Syzygium jambos* trees, each with two levels were applied to the plots in a two-way factorial design: chemical type and physical treatment method.

The chemical types used were: **T**-Tordon Brush killer ™ Dow Agro Sciences: 100g/litre Picloram salt or **R**-Roundup/Roundup Renew/ Glyphosate Gold™: 36% Glyphosate. The chemicals were mixed with water and indicator dye (blue dye to aid visualisation of the area treated) of methyl blue (Big Dye™, PinePac, New Zealand) at the recommended rates (1 kg per 150 litres + indicator dye) and applied as a high volume spray to all seedlings and saplings and at more concentrated rates (70% chemical) for stems.

The two physical treatments applied were: 1.**Cut** treatment, where *Syzygium jambos* trunks were cut as close as possible to ground level with chainsaws, and the branches and trunks were removed from the experimental site. One of the two herbicide solutions described above was

applied to the entire cut surface, and all bark below the cut surface to ground level. This method is referred to as the "cut treatment".

The second physical treatment: 2.**Frill** treatment Involved frilling or removing the bark from a collar at the base of standing *Syzygium jambos* trees, the sapwood was exposed from the entire circumference of the tree, using small hatchets and knives. Efforts were made to include notches which penetrated further into the stems where stem diameters were greater than 20cm. A herbicide solution was applied to all the exposed sapwood and into the notches. This method is referred to as the "frill treatment". *Syzygium jambos* remained in situ with this treatment (Plate 2).

The total number of 10x10m subplots treated was eight-four. Forty-eight subplots were treated with Tordon, of which 32 were cut and 16 frilled. Four subplots were treated with Tordon and Diesel, of which, 2 were cut and 2 were frilled. Four subplots, with both Tordon and Roundup, 2 of which were frilled and 2 cut. Twenty-eight subplots were treated with Roundup; 14 cut and 14 frilled (Table 3.1).

Table 3.1 Treatments applied to the 84 experimental *Syzygium jambos* 10x10m subplots

	Tordon	**Tordon & Diesel**	**Tordon & Roundup**	**Roundup**	**Total**
Cut	32	2	2	14	**50**
Frill	16	2	2	14	**34**
Total	**48**	**4**	**4**	**28**	**Σ=84**

Ideally all treatments should be balanced, but chemical delivery to the island was delayed in 2003 and more subplots than was originally intentioned were treated with Tordon. One plot, White Seed (plot 11) with four subplots received a mixed chemical treatment with both Roundup and Tordon applied, and another plot Flatlands Camp (plot 1) received a mix of both Tordon and Diesel. The Cut and Frill treatments also should have been balanced but in certain subplots where just one or two large adults remained, these were removed by chainsaw operators.

Plate 2. *Syzygium jambos* frill treatment where bark is removed from the circumference of the stem at the base of the tree and chemical applied to exposed sapwood. Note the resprouting from the base and the callus tissue above the exposed sapwood which is relevant to the discussion (Section 3.4.1).

All saplings in each subplot were cut with a machete and removed from the site with herbicide applied as a high volume spray to the cut surfaces. The seedlings in each subplot were treated in-situ with a high volume spray. In inaccessible areas, chemical was applied with 15 litre knapsack sprayer (Solo®, PinePac, New Zealand) and in sites accessible to the road a tractor mounted high volume sprayer (SHURflo® Diaphragm pump, PinePac, New Zealand) was used.
As the density of *Syzygium jambos* seedlings was extremely high, a total spray of the ground flora was required, and while efforts were made to avoid any native plants while the operators were spraying, there was no guarantee that herbicide drift would not affect them. Experimental subplot treatments were carried out from August (2003) and continued to May (2004), data recorded from all the treatments applied were recorded during April and May 2005. The selected treatments were assigned to the eighty-four permanent subplots in twenty one plots chosen.

3.2.3. Seed bank sampling

To access the content of the seedbank in *Syzygium jambos* forest on Pitcairn Island, investigations into the proportion of *Syzygium jambos* and native species that occur in the seed bank were initiated before herbicide application to plots.

Soil seed bank samples included the litter layer to ensure any transient members of the seed bank were included. The samples were collected using a trowel (3.5cm diameter to a depth of 10cm), 30 samples were taken from within a plot. The samples were bulked in the field and mixed thoroughly before working through by hand to remove stones, roots and coarse plant debris and then placed in 35.5 x 21.5 x 6.0 cm seed trays, (1 tray per site) and covered with a thin layer of commercial seed compost (Garden Care Compost; PinePac, NZ).

The seed trays, including controls (containing only compost), were randomly arranged on a capillary bed in Pitcairn Island nursery and watered daily for two months. The geminated seedlings were identified to species level where possible using Göthesson (1997) and individuals counted after the three-month period (March-May 2004) when emergence had virtually ceased. Seedling numbers were then expressed per unit volume of soil.

3.2.4 Data handling and analysis

Data matrices and calculations were compiled and performed using MS EXCEL. Graphs were also produced in MS EXCEL and standard errors calculated and manually assigned to the graphs. Nonmetric multidimensional scaling (NMS) was carried out in PC-ORD Version 4.01. Sorenson (Bray-Curtis) distance measure was used for the ordinations and the data were run on the "slow and through" setting (McCune & Mefford 1999). The ordination diagrams using the results obtained were constructed in MS EXCEL. The non-parametric Kruskal Wallis was carried out in SPSS Version 12.0.1 for Windows. ANOVAs were also performed in GMAV5 for Windows (Underwood *et al.* 2004).

3.3 Results

3.3.1 *Syzygium jambos* treatment and control

Seedling, sapling and adult *Syzygium jambos* were treated chemically during 2003 and 2004, and mortality was assessed in 2005. In total, 1792 adult trees were treated the majority of which were in the 10-15cm (734 individuals) in diameter, with some very large individuals with diameters in each of the categories 160-170cm, 171-180cm, 181-200cm and 201-300cm (Figure 3.1). An estimated 400,848 seedlings and 51,240 saplings were also treated based on mean seedling and sapling density per metre calculations (Chapter 2).

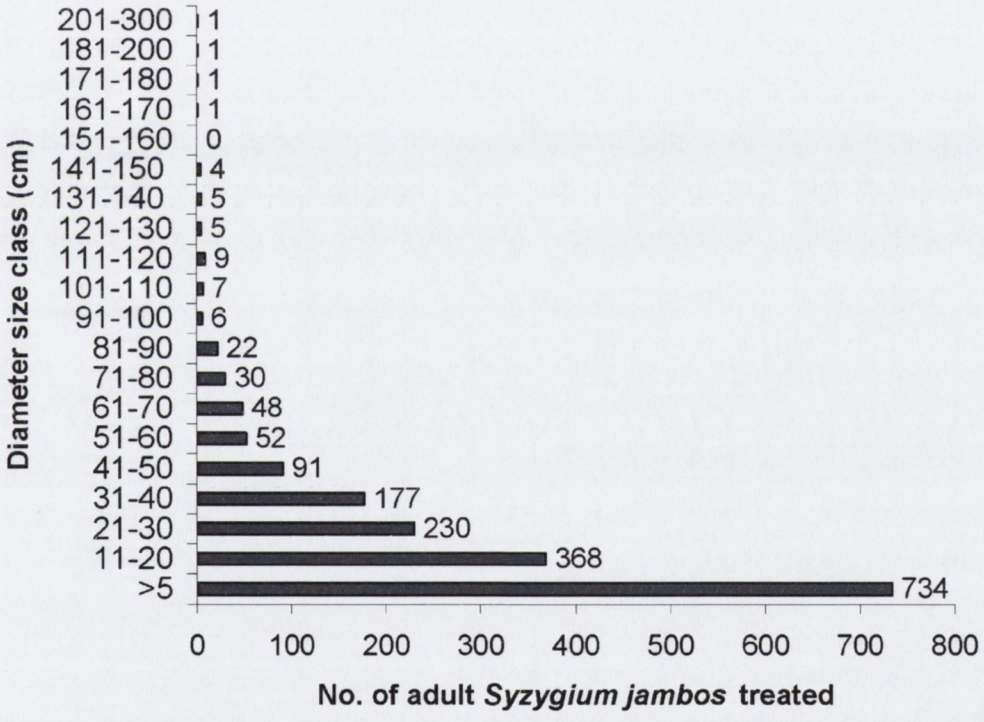


Figure 3.1 Diameter profile of adult *Syzygium jambos* trees chemically and physically treated.

In 2005 the mean *Syzygium jambos* mortality[7] rate for adult plants across all physical and chemical treatments was 97.9(±3.2)%. *Syzygium jambos* mortality with each of the chemical and physical treatments is outlined in Figure 3.2 Syzygium *jambos* adult plants treated chemically with Tordon and physically cut, had a mortality of 97.5(±3.0)% and plants treated with Tordon and physically frilled had a slightly lower mortality rate which was 96.57(±4.48)%. Chemical treatment with Roundup and physically cut had higher mortality than those treated with Tordon. Using Roundup

[7] **mortality**- figures based on number of adults that were defoliated with no resprouting from the base of trunks.

and the physical cut treatment the mortality was 98.71(±2.6) % and mortality with Roundup and the physical frill treatment had the highest mortality at 98.86(±1.4)%.

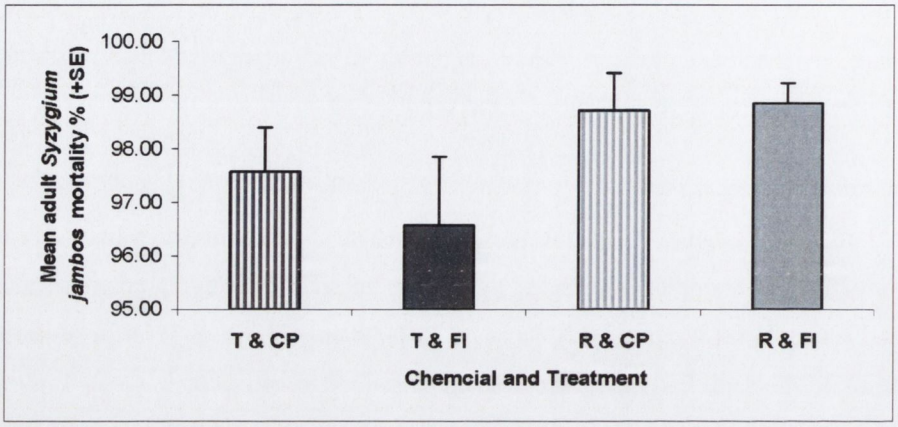


Figure 3.2 Mean (+SE) adult percentage mortality of *Syzygium jambos* with the different subplot treatments. Chemicals: Tordon (T) and Roundup (R) and physical treatment method: Cut (CP) and Frill (FI).

In order to fulfil the requirements of balance and independence for statistical analysis in GMAV, fourteen subplots were drawn from each treatment. The data failed to satisfy the criteria of Cochran's test and transformations did not improve normality. A non-parametric Kruskal Wallis (K) test was carried out on these data. There was no significant difference in mortality with *Syzygium jambos* physical treatment method (p=0.649, Table 3.2) or with chemical used (p=0.166,Table 3.3).

Table 3.2 Kruskal-Wallis test of treatment method on mortality of *Syzygium jambos*

Treatment	*N*	*Mean rank*	*K*	*P*
Cut	28	25.64	1.893	0.166 ***ns***
Frill	28	31.36		
Total	56			

ns=not significant

Table 3.3 Kruskal-Wallis test of chemical applied on method on mortality of *Syzygium jambos*

Chemical	*N*	*Mean rank*	*K*	*P*
Tordon	28	29.45	0.208	0.649 ***ns***
Round-up	28	27.55		
Total	56			

***ns*=not significant**

No saplings of *Syzygium jambos* were found resprouting during fieldwork in 2005 or in 2006 by the project manager. The seedling mortality rate for 2005 was high in all chemical and physical treatments. *Syzygium jambos* seedling mortality for 2005 is presented in Figure 3.3 and there were no reported seedlings in the treated subplots in 2006. Marginally higher mortality rates of seedlings were obtained when adult *Syzygium jambos* was removed from the subplots with the cut treatment. The lowest mortality rate was found in subplots which were treated with Tordon and frilled, though none of these differences were significant (Figure 3.3).

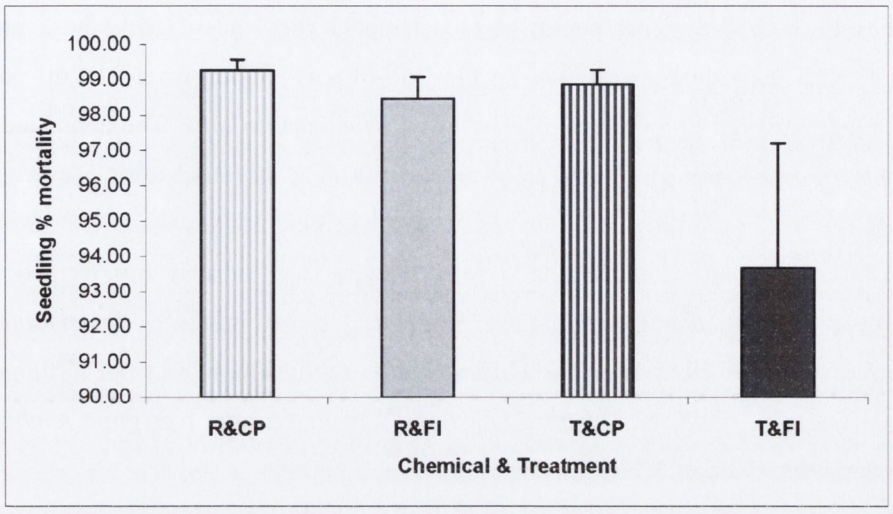


Figure 3.3 Mean percentage mortality of *Syzygium jambos* seedlings in the treated subplots. Chemicals: Tordon (T) and Roundup (R) and physical treatment method: Cut (CP) and Frill (FI).

A number of the adult *Syzygium jambos* plants treated started to re-sprout in 2006. The estimated percentage re-sprout for the various treatments were calculated by the Conservation officer (J. Warren *pers. obs.*) and the Project manager (C. Warren *pers. obs.*) and are reported Figure 3.4. The mortality rate for 2006 dropped to 75% with approximately 450 of the adult trees showing signs of resprouting. The majority of trees resprouting were found to be those that were chemically treated with chemical Round-up.

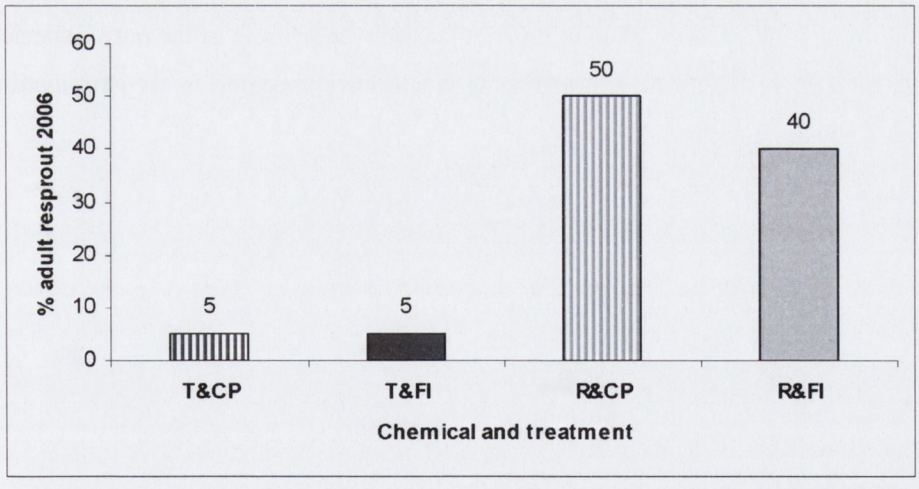


Figure 3.4 Estimated percentage re-sprout of *Syzygium jambos* adults treated with chemicals: Tordon (T) and Roundup (R) and physical treatment method: Cut and painting (C) and Frill (FI) in 2006.

3.3.2 *Syzygium jambos* forest and the soil seed bank

The mean number of species which emerged from the seedbank samples from sites was 5.1 (±1.45) and the number of species found in sites ranged from 3 to 8 species (Table 3.4). *Syzygium jambos* emerged in the germination trial from every plot. However, it was not the most abundant species in the seedbank. The most abundant species to emerge from the seedbank were *Oxalis corniculatus* (60 individuals; 21.1%) followed by *Eleusine indica* (56 individuals; 19.72%).

Seed densities of each emergent species were calculated for $1m^{-3}$ of soil, these are presented in Table 3.4, with individual species values for $1m^{-3}$ of soil ranging from 218 m^{-3} for *Desmodium tortuosum* and *Kyllinga brevifolia* to 727 m^{-3} for *Oxalis corniculatus*. The abundance and range of species in the seedbank had more in common with species found after *Syzygium jambos* treatments (Section 3.3.3) and did not match the vegetation found at sites when they were initially surveyed in 2003. This may indicate that weedy species were present and formed a seedbank when land was initially abandoned, before *Syzygium jambos* invasion. *Desmodium tortuosum* appeared only from the seedbank of soil from Site 9 Devils Elbow and the *Kyllinga* species which emerged from the seedbank were subsequently only found after *Syzygium jambos* control treatment in damp sites (Section 3.3.3).

Fern species were absent from the seedbank, this result though initially surprising, given that *Syzygium jambos* is regularly found in association with *Nephrolepis cordifolia*, *Nephrolepis biserrata*, and *Nephrolepis hirsutula*, is perhaps not, as it is thought that soil conditions in *Syzygium jambos* forest are not conducive to the preservation of spores or the germination conditions in the nursery were not suitable for them.

Two shrubby tree species found regularly associated with *Syzygium jambos; Pandanus tectorius* and *Cordyline fruticosa* did not appear in the seedbank, suggesting that these species are rarely found in the seedbank. No native plant species emerged from the soil seed bank experiment which indicates the serious nature of *Syzygium jambos* invasion and its effects not only on the extant native flora but on the future flora. Though it is not known whether any of the native species form persistent seedbanks, it was likely though that some would have appeared in the germination trial if they were even transient members.

Table 3.4 Soil seed bank results: numbers of individuals emerging from seed trays from soil from each plot. Abbreviations: Syz-Nep (*Syzygium jambos-Nephrolepis* spp.); Syz-Pan (Syzygium *jambos-Pandanus tectorius*) *Syz-Cor* (*Syzygium jambos-Cordyline fruticosa*); Syz-Opi (*Syzygium jambos-Oplismenus hirtellus*) & Syz-Cya (*Syzygium jambos–Cyathea medullaris*) & Syz-Psi (*Syzygium jambos-Psilotum nudum*) were the two most dominant species in each plot before *Syzygium jambos* control treatments.

Plot	Dominant vegetation	*Canna indica*	*Chamaesyce hirta*	*Desmodium tortuosum*	*Elusine indica*	*Kyllinga brevifolia*	*Kyllinga nemoralis*	*Lantana camara*	*Oxalis corniculatus*	*Sonchus oleaceus*	*Sorghum sudanense*	*Syzygium Jambos*	Unidentified Grass Seedlinds	Total No. of species
1	Syz-Nep	-	2	-	4	-	1	-	7	2	2	1	-	7
5	Syz-Nep	-	-	-	18	-	-	1	4	2	-	2	-	5
6	Syz-Nep	-	-	-	4	-	-	-	2	3	3	3	-	5
7	Syz-Nep	-	-	-	2	-	-	1	13	3	-	4	-	5
11	Syz-Nep	-	-	-	-	-	-	-	2	-	-	3	2	3
12	Syz-Nep	-	-	-	3	-	-	-	-	-	2	2	-	3
13	Syz-Nep	2	-	-	-	-	-	-	2	-	-	2	-	3
16	Syz-Nep	-	-	-	3	-	-	4	1	2	2	1	-	6
18	Syz-Nep	2	1	-	2	-	-	3	2	3	2	1	-	8
2	Syz-Pan	-	-	-	1	1	2	-	-	-	5	2	-	5
3	Syz-Cor	-	-	-	1	-	-	1	-	-	-	1	-	3
4	Syz-Cor	-	-	-	-	-	-	9	2	2	2	3	-	5
9	Syz-Cor	-	-	1	2	-	1	2	5	-	3	8	4	8
10	Syz-Cor	-	-	-	5	-	-	1	4	1	-	4	-	5
14	Syz-Cor	-	-	-	3	-	1	3	1	-	2	1	-	6
15	Syz-Cor	2	-	-	2	-	4	8	1	-	-	3	-	6
17	Syz-Cor	1	1	-	-	-	-	-	1	-	-	1	5	5
19	Syz-Cor	-	-	-	2	-	-	2	2	-	2	1	-	5
8	Syz-Psi	-	-	-	-	-	-	1	9	-	-	6	3	4
20	Syz-Opi	-	-	-	3	-	-	3	1	-	2	1	-	5
21	Syz-Cya	1	-	-	1	-	1	-	1	1	-	3	-	6
	Total Emerged	**8**	**4**	**1**	**56**	**1**	**10**	**39**	**60**	**19**	**27**	**53**	**14**	**284**
	Seed m^{-3}	**350**	**291**	**218**	**764**	**218**	**364**	**655**	**727**	**461**	**536**	**551**	**764**	**5900**

3.3.3 Secondary weed invasion following *Syzygium jambos* experimental control

The percentage of weedy plant species cover in each of the treated plots was recorded in 2005. In fifty of the eighty-four subplots, *Syzygium jambos* was cut and removed and the stumps painted with chemical, in the remaining thirty-four subplots the bark was frilled from the base of the tree and the chemical sprayed onto the exposed sapwood. The percentage weed incidence in plots with different physical treatments was very different, as was the weedy species regenerating in each physical treatment method.

The average percentage weed cover in the cut plots (CP) was 71.1(±20.7)% and percentage weed cover in frilled plots (FI) was much lower at 18.0(±12.6)% .The data were $\sqrt{(x+1)}$ transformed to satisfy conditions of normality (C=0.53, ns) and balanced ANOVA in GMAV. The difference in percentage weed cover was found to be significant between treatments (p=0.0001, Table 3.5 & Figure 3.5).

Table 3.5. ANOVA of percentage weed cover in subplots under the two different *Syzygium jambos* treatments.

Source	*SS*	*DF*	*MS*	*F*	*P*
Const	309.5737	1	309.5737	164.86	0.0001
Treatment	123.9358	66	1.8778		
Total	433.5095	67			

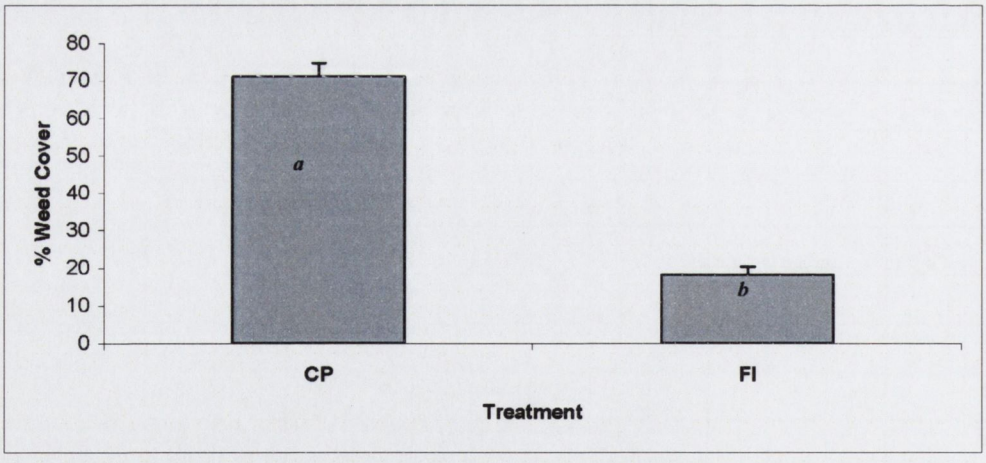


Figure 3.5 Percentage weed cover in plots with two different adult *Syzygium jambos* physical treatment methods. The letters *a* and *b* indicate significant differences at α ≤0.001 in a Student Newman Keuls post hoc test. ($F_{[1,67]}$ 164.86; p<0.001; n=68)

To fully understand the differences in weedy species cover in the treated plots an NMS ordination was conducted using the Domin species cover values for the 23 common weed species found in 84 plots. Forty runs with real data and fifty with randomized data recommended a two-dimensional solution (Figure 3.6) after the initial runs (Table 3.6). The best two dimensional solution was used as the starting position for the final ordination. The final stress value for the two-dimensional

solution was 19.33 (Legendre & Legendre 1998) which was significantly lower (p=0.0196) than would be expected by chance.

Table 3.6. Mean Stress and *p values for an NMS ordination initial run dimensions for the dataset of *Syzygium jambos* experimental plot treatments and weed species found regenerating after one year of planting.

Dimension	Mean Stress in real data Stress in randomized data, 40 runs	Mean Stress in randomized data. Monte Carlo test, 50 runs	p*
1	44.98	50.39	0.0196
2	22.33	31.45	0.0196
3	19.86	24.12	0.0196
4	18.32	19.87	0.0196
5	19.96	21.13	0.0196
6	18.82	17.17	0.0392

*p = proportion of 50 randomized runs with stress < or = observed stress i.e., p = (1 + no. permutations <= observed)/ (1 + no. permutations)

The first dimension reflects the difference in the weedy species regenerating in the two physical experimental treatments; Cut and Frill. The transition is clear from cut plots clustering on the left or negative axis of the ordination to frilled plots clustering on the right or positive axis (Figure 3.6). The weed species positively correlated with Dimension 1 include *Syzygium jambos* seedlings (r = 0.76) and *Oxalis corniculata* (r = 0.66); these species were the dominant weedy species found in frilled plots (Figure 3.6).The species commonly found in cut plots, for example, Conyza *bonariensis* (r = -.0.53), *Lantana camara* (r = -0.76) *Paspalum conjugatum* (r = -0.54) *Sorghum sudanense* (r = -0.70) and *Verbena bonariensis* (r = -0.66) were all negatively correlated with Dimension 1 (Figure 3.6). Dimension 2 also separated the physical plot treatments with mostly negative correlations for species in cut plots and positive correlations for species in frilled plots (Figure 3.6).

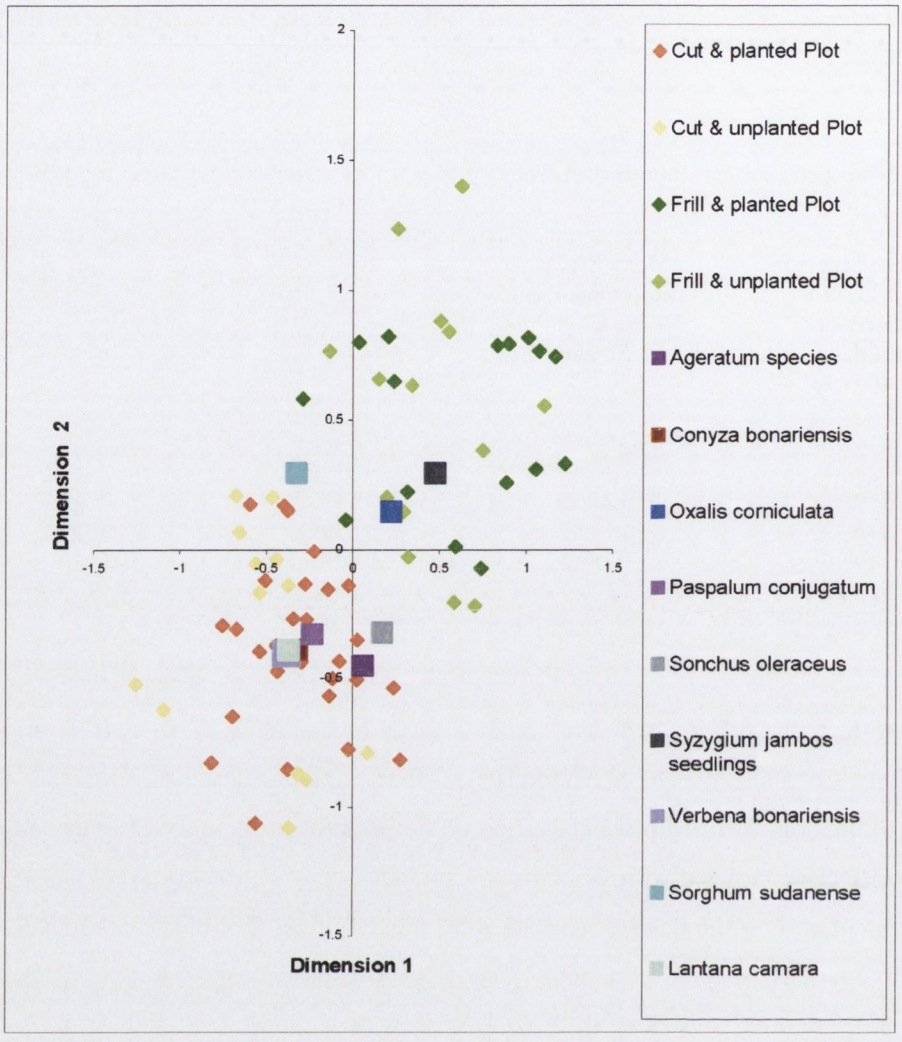


Figure 3.6 NMS ordination of species cover in *Syzygium jambos* cut and frilled plots. Ordination stress was 19.33. Species found regenerating in each treatment are highlighted with square symbols and plot treatments highlighted by diamond symbols.

The findings demonstrated that there was a marked difference in the weedy species which invaded plots after *Syzygium jambos* control efforts. Distinct weedy species assemblages observed with each treatment are highlighted in Plate 5 and 6. *Lantana camara, and Conyza bonariensis* were common in plots where *Syzygium jambos* was cut (Plate 3) and *Oxalis corniculata* was commonly found in frilled plots (Plate 4). These species were all found occurring in sites during the seedbank experiment (Table 3.4). These results further demonstrate the relevance of small scale seedbank study experiments as a predictor for determining the vegetation after invasive species control.

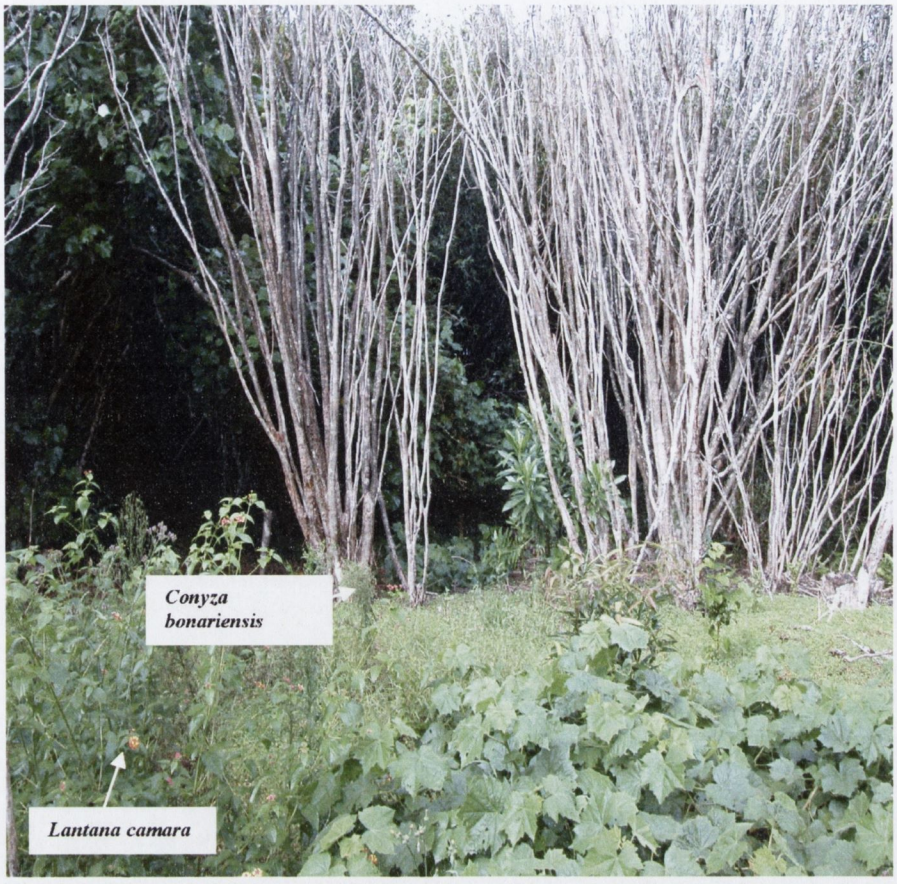


Plate 3. Site 1 Flatlands Camp showing subplot 1A with weedy invasion after *Syzygium jambos* adults were physically cut and removed from the plot. On the left is *Lantana camara* with orange flowers and the tall plumes of *Conyza bonariensis* in the background.

Plate 4. Site 1 Flatlands Camp showing subplot 1C with weedy invasion after Syzygium jambos adults were physically frilled and left in-situ. In the foreground is *Oxalis corniculatus* which was dominant in Frilled plots.

3.3.4 Natural regeneration following *Syzygium jambos* control treatment

Fifteen native and introduced species found in native woodland communities on Pitcairn were found naturally regenerating in *Syzygium jambos* treated subplots (Figure 3.7). *Glochidion pitcairnense* (native tree) was found most frequently, regenerating in both cut (21.74%) and frilled (22.22%) subplots.

Other native tree and shrub species which were found regenerating were *Cyclophyllum barbatum* in both cut and frilled subplots in low percentages, *Hibiscus tiliaceus* in high percentages in cut subplots and low percentages in frilled subplots, *Homalium taypau,* low percentage in both cut and frilled subplots, *Pandanus tectorius* in high percentages in frilled subplots, *Xylosma suaveolens* which was only found in frilled subplots and *Cocus nucifera* which was only found in cut subplots (Figure 3.7).The native ferns *Davallia solida*, *Pneumatopteris costata* var. *hispidula* appeared to regenerate more frequently in frilled subplots but some were found in cut subplots. Other species of native ferns; *Doodia media*, *Nephrolepis cordifolia* and *Phymatosorus scolopendria* were only found regenerating in frilled subplots (Figure 3.7).

Species introduced to the island by the Polynesians were also found regenerating in subplots with *Cordyline fruticosa* found in both cut (21.74%) and frilled (2.78%) subplots and *Thespesia populnea* also found in a similar percentage of both cut (6.52%) and frilled (5.56%) subplots (Figure 3.7). *Pinus sp.* (possibly *caribaea*) was found to be regenerating in cut (17.39%) subplots (Figure 3.7). *Pinus caribaea* however, is now considered invasive throughout French Polynesia (J-Y. Meyer *pers. comm.*).

The native and introduced species common in native forest communities which were found regenerating in the treated subplots occurred within 50m of the treated subplots as fruiting or sporing adults. These results highlight the importance of proximity to native vegetation for natural regeneration following *Syzygium jambos* control.

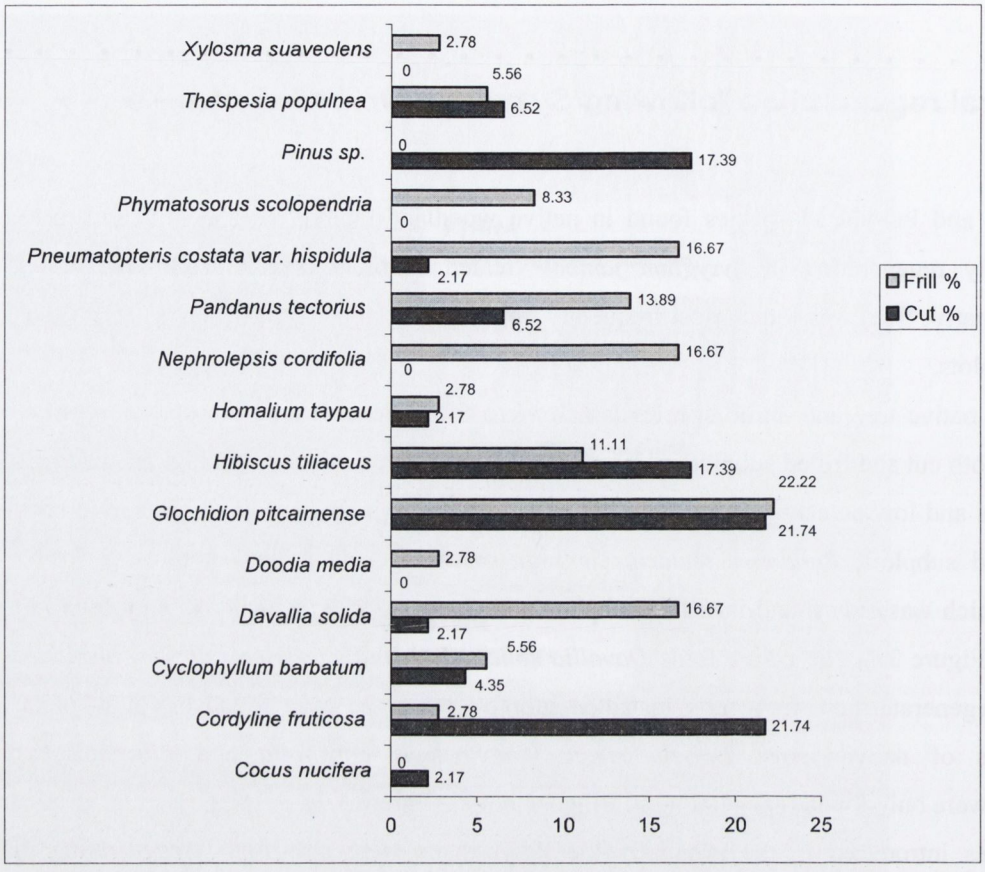


Figure 3.7 Percentage of *Syzygium jambos* cut (46 subplots) and frilled (34 subplots) plots with native and introduced species naturally regenerating.

Species: Cocus nucifera (native tree); Cordyline fruticosa (tree-Polynesian introduction; Cyclophyllum barbatum (native shrub); Davallia solida (native fern); Doodia media (native fern); Glochidion pitcairnense(native tree); Hibiscus tiliaceus (native tree); Homalium taypau (native endemic tree); Nephrolepis cordifolia (native fern); Pandanus tectorius (native tree); Pneumatopteris costata var. hispidula (native fern); Phymatosorus scolopendria (native fern); Pinus sp (introduced tree potential invasive); Thespesia populnea (tree-Polynesian introduction); Xylosma suaveolens (native tree).

3.3.5. Syzygium jambos treatment and the cost of control

The cut physical treatment took more time to treat each *Syzygium jambos* individual. The average time taken to chemically and physically cut-treat a plot of $400m^{-2}$ was 106 person hours. The time was used over three days consisting of two days with 6 people: 3 people cutting *Syzygium jambos* with chainsaws and 3 people clearing away the timber. An additional half day with 2 people (8 hours) was required for chemical herbicide application. Herbicide application was easier in cut sites when *Syzygium jambos* timber was removed. The work was very labour intensive, difficult and dangerous on steep slopes. An additional time of 2 hours was also allowed for follow-up inspection which consisted of checking that each individual adult tree was chemically treated with herbicide.

The frilling control treatment with herbicide application took less time in physically treating each individual *Syzygium jambos* tree, the average time taken to treat a $400m^{-2}$ plot was 66 person hours. The time was spent over two days which consisted of three people using knives and hatchets to remove the bark and a chainsaw operator making cuts into trunks when diameters were over 50cm. Chemical herbicide application took longer in frilled plots as it was more difficult for operators to move around the plot. A full day with two people was required, 16 hours per $400m^{-2}$ site. Follow up treatment and inspection was similar to cut plots with 2 hours allocated per $400m^{-2}$ plot. Frilling is also labour intensive but much less than with cut method and it is less dangerous for operators on steep slopes. The labour costs in Pitcairn were based on New Zealand minimum pay rates (2004) with $100 NZ dollars charged as a daily rate for an 8 hour day or $12.50 NZ an hour. The equivalent rate in Euro is €54.86 per 8 hour day (Table 3.7).

Table 3.7 Labour cost variables for *Syzygium jambos* control outlining person hours required cost using two different physical and chemical treatments

Labour cost for treating *Syzygium jambos* *400m^{-2} plot	**Physical Treatment**	**Chemical Treatment**	**Follow-up Inspection**	**Total**	**$Euro Total Cost**
Cut treatment Person hours	96	8	2	**106 person hours**	**€726.89**
€ Euro	658.32	54.86	13.71		
Frill treatment Person Hours	48	16	2	**66 person hours**	**€452.1**
€ Euro	328.8	109.6	13.7		

* 25 Sites = 1 ha

The capital start-up cost of the cut method totalled €2125.03 Euro; which included chainsaws, safety boots, helmets, and gloves. The start up cost of the frill method was less expensive with start-up costs of €905.93; which requires a chainsaw, gloves, helmet, safety boots, hatchets and knives (Table 3.8 and Figure 3.8).

Table 3.8 Costs associated with the physical control treatment of *Syzygium jambos*

Costs for physical control treatment of *Syzygium jambos*	Cut treatment €Euro		Frill treatment €Euro	
Capital	**Cost**	**No. required**	**Cost**	**No. required**
Chainsaw	545	3	545	1
Safety boots	55.70	6	55.70	3
Helmet	65.83	3	65.83	1
Gloves	30	3	30	3
Hatchet	7	0	7	2
Knife	12	0	12	2
Total capital cost	**2125.03**		**905.93**	
Capital over 5 years	**425.00**		**181.18**	
Recurrent Cost				
Gloves	30	3	30	3

*Costs from PinePac, 2-6 Airport Road, Whenuapai, Auckland, December 2006

The capital cost of chemical application of both methods is similar with knapsack sprayer (15 L) or optional tractor mounted sprayer which would cost an additional €2518. The cost of chemical safety suits, safety respirators, rubber gloves, indicator dye and herbicide costs for Round-up 20L, which treats 1ha and Tordon 1L, which can treat 3 ha (Table 3.9 and Figure 3.8)

Table 3.9 Costs for Chemical control of *Syzygium jambos*

Costs for Chemical control of *Syzygium jambos*	Cut treatment €Euro		Frill treatment €Euro	
Capital cost	**Cost**	**No. required**	**Cost**	**No. required**
Knapsack sprayer	105.65	1	105.65	1
Safety Suit	10	2	10	2
Safety Respirator	35	2	35	2
Total capital cost	**195.65**		**195.65**	
Capital cost over 5 years	**39.13**		**39.13**	
Recurrent Cost				
Rubber gloves	4	2	4	2
Round-up	110	1	110	1
Tordon	50	1	50	1
Indicator Dye	95.50	1	95.50	1

*Costs from PinePac, 2-6 Airport Road, Whenuapai, Auckland, December 2006

The total cost of the two physical control methods with follow up chemical herbicide application using Round-up was €18,939.88 for the Cut treatment and €11,826.31 for the frill treatment. The labour costs associated with the frill treatment are much lower than the cut treatment and the start up costs of the frill treatment are also much lower than the cut treatment (Table 3.10 Figure 3.9).

Table 3.10 Costs of control for *Syzygium jambos* per hectare

Capital Costs (over 5 years)	**Cut/Tordon**	**Cut/Roundup**	**Frill/Tordon**	**Frill/Roundup**
Physical equipment	425.00	425.00	181.18	181.18
Chemical equipment	39.13	39.13	39.13	39.13
Recurrent Costs	**Cut/Tordon**	**Cut/Roundup**	**Frill/Tordon**	**Frill/Roundup**
Labour	18,172.25	18,172.25	11,302.50	11,302.50
Physical treatment	90	90	90	90
Chemical treatment	153.50	213.50	153.50	213.50
Cost to treat 1ha using local labour	**18,879.88**	**18,939.88**	**11,766.31**	**11,826.31**
Cost to treat 1ha using free /"volunteer" labour	**707.63**	**767.63**	**463.81**	**523.81**

The labour costs associated with the frill treatment (€11,302.50) are also much lower than the cut treatment (€18,172.25) per hectare and the start up costs of the frill treatment are also much lower than the cut treatment (Table 3.10 and Figure 3.9).

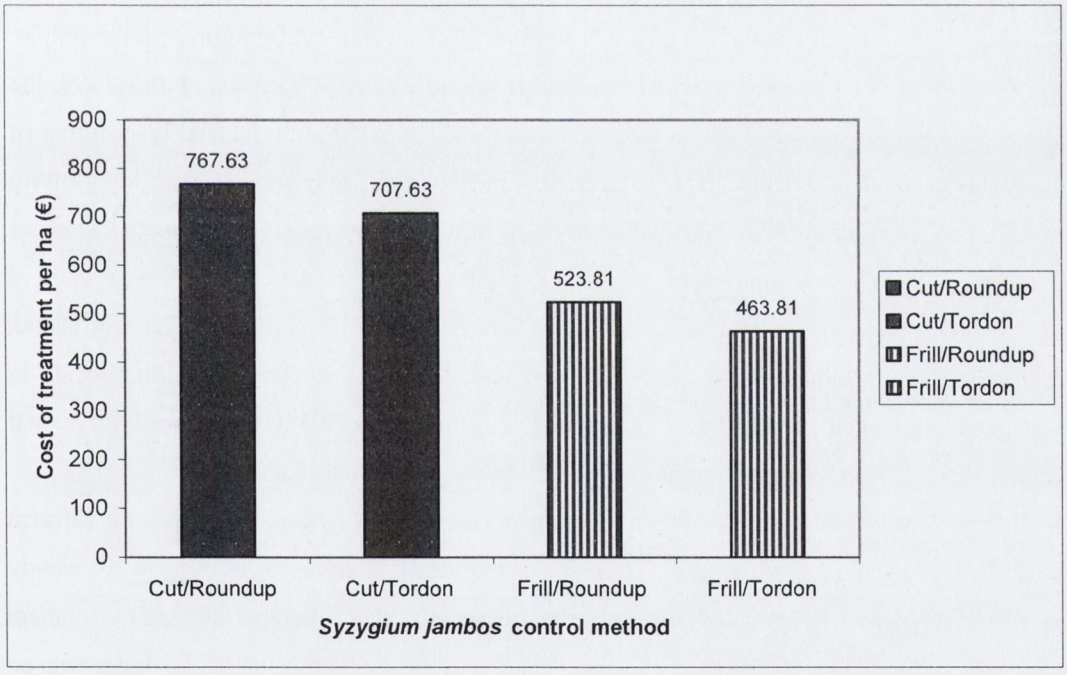


Figure 3.8 The cost of physical and chemical control of *Syzygium jambos* per hectare (excluding labour costs and the capital costs were spread over a 5 year period).

3.4. Discussion

3.4.1 Control treatment effectiveness

Syzygium jambos mortality was assessed for seedling, sapling and adult plants in 2005 and was found to be very effective with little difference between the effectiveness of either the chemical or physical treatment method applied. However, the project managers on Pitcairn Island (Carol & Jay Warren) have noted that mortality rate for adult *Syzygium jambos* had dropped to 75% in 2006 (with approximately 450 of the 1792 of the adult plants treated resprouting). Plots treated with Round-up, and to a lesser extent plots treated with Tordon had resprouting adults. The initial effectiveness of the chemical and physical treatments was misleading. The development of callus tissue burls noted on frilled trees in 2005 seemed to produce much of the re-sprouts that were noted in 2006. Individuals treated with large diameters also required second and even third applications of herbicide.

Delayed resprouting can be a feature of invasive species control programmes, for example, *Lonicera maackii* in the eastern U.S. was treated with Round-up in 1998, at the end of 1999, the above ground mortality was 99%, which was reduced to 94% in 2000 with delayed resprouting of individuals (Hartman & McCarthy 2004). Herbaceous grass species may require even longer term monitoring with two-four sprays a year for a period of three years required to eradicate *Cenchrus ciliaris* (Buffel grass) on Airlie island western Australia (Dixon *et al.* 2002). Five years of treatment with herbicide and pulling was required to control *Cenchrus echinatus* at Laysan Island, Central Pacific Ocean (Flint & Rehkemper 2002). The requirement for long term monitoring in invasive species management has been highlighted by Blossey (1999) and the need for repeat surveying of treated areas for surviving individuals by Mack & Lonsdale (2002).

Chemical treatments were mainly successful in controlling *Syzygium jambos* on Pitcairn Island. While cutting and applying herbicide is one of the most widely used procedures for woody invasive plants (Ogden & Rejmánek 2005; Tye *et. al* 2002; Flint & Rehkemper 2002; Environment Australia 1997; Motooka *et al.* 2002), it was found to be extremely labour intensive, dangerous on steep slopes as well as expensive. Secondary weedy species invasion following *Syzygium jambos* cut treatment was also a problem on Pitcairn Island. The frill method is becoming more widely used (Johansson 1985, Franz & Keiffer 2000) it may be the best method for control of *Syzygium jambos* in the future on Pitcairn Island, being less labour intensive and dangerous, with lower costs and a marked reduction of secondary weedy species invasion. Advances in the technology such as the development of chemical capsules for use with lance guns such as the EZJECT lance (Odum Processing Engineering Consulting Inc., Waynesboro, Mo, U.S.A) (Franz & Keiffer 2000; Hartman *et al.* 2004) would no doubt further improve the safety, cost and effectiveness of *Syzygium jambos* control efforts on Pitcairn Island in the future.

Seedling and sapling mortality rates were also high showing the suitability of both herbicides used, Tordon and Round-up, for *Syzygium jambos* control. The application rate and application timing also appear to be successful as the mortality results stayed similar for these groups during 2005 and 2006. With control efforts reinvasion must be prevented and if there is the continued threat of re-invasion (Mack & Lonsdale 2000) efforts must be made to repeatedly survey a treated area and they suggest a time frame of ten years or more.

3.4.2 Composition of the soil seed bank in *Syzygium jambos* forest

The soil seedbank in *Syzygium jambos* forest does not contain or maintain members of the species found in native forest communities, and this will make the restoration of native forest following removal of *Syzygium jambos* more challenging. The top five weedy species that emerged from the trails conducted were *Oxalis corniculatus*, *Eleusine indica*, *Lantana camara*, *Sorghum sudanense* and *Sonchus oleraceus,* these may be maintained in the seedbank because they are long-lived and were the common weeds during the cultivation phase of much of the centre of the island before *Syzygium jambos* invasion.

The native shrubby species found commonly associated with *Syzygium jambos* were *Cordyline fruticosa* and *Pandanus tectorius*. They have seed larger than 0.5mm and were absent from the seedbank trails conducted. It has been noted by other authors that larger seed do not form persistent (> 5years) seedbanks and larger seed are more likely to form a "transient seedbank" which means they are confined to upper layers only for a short period which is generally around one year. Large seeds generally do not penetrate into the deeper layers of soil (Baker *et al.* 1996) this may explain the absence of *Cordyline fruticosa* and *Pandanus tectorius* from the soil seed bank.

It was however, noted that there can often be a lack of correspondence between standing vegetation of an area and the seed bank composition in forests (Roberts 1981; Augusto *et al.* 2001; Cohen *et al.* 2004). Formation of a transient as opposed to a permanent seedbank is common for dry tropical woody forest species (Ogden 1985, Demel & Granström 1995, Drake 1998) and tropical forest seeds are in many cases short-lived (Garwood 1989) which may explain the lack of native woody forest species in the seedbank of *Syzygium jambos* forest. The timing of the investigations may also be of significance these trials were carried out from March-May in 2004 which in the southern hemisphere equates to our northern hemisphere autumn in which potential transient members may not have had time to accumulate from the seedrain.

Timing and perhaps additional time required for spore germination may explain the absence of the native fern species *Nephrolepis cordifolia, Nephrolepis biserrata* and *Nephrolepis hirsutula* found commonly associated with *Syzygium jambos* in invaded forest. Fern spores are also adapted for long range dispersal which can diminish the chance of seedbank formation (Klinkhamer *et al.* 1987).

The seed densities from the germination trail of seedbank samples from *Syzygium jambos* forest on Pitcairn Island ranged from 218 seeds per m^{-3} to 764 seeds per m^{-3}. *Syzygium jambos* was found at a density of 551 m^{-3}. These values are comparable to values found by Baker *et al.* (1991) who noted that the majority of species that exist in the seedbank have seed densities below 500 m^{-3}.These values however, contrast with the seed density of invasive *Celastrus orbiculatus* (Oriental bittersweet) in Ohio which had zero mean density in seedbank trials suggesting it was absent from the seedbank (Ellsworth *et al.* 2004).

The results from these trails suggest that *Syzygium jambos* forms part of the seedbank, whether it is a persistent or transient member is not clear, but this feature will make the elimination of *Syzygium jambos* more difficult (Simberloff 2001). The lack of native forest species in the soil bank suggests that the alien weedy species found are more favourably placed than native species to increase in abundance once *Syzygium jambos* is controlled and possibly indicates that *Syzygium jambos* has spread into areas which were formerly cultivated and cleared in the past.

3.4.3 Secondary invasion by weedy species following *Syzygium jambos* control efforts.

The secondary invasion of treated *Syzygium jambos* plots with weedy grass and herbaceous species was predicted from experiments into the composition of the seedbank in the trial plots. The suite of species found in the seedbank experiment (Table 3.3) mostly matched the vegetation found in the trial plots during 2005 (Section 3.4.2).

In many control programmes success in eradicating one species can lead to the explosion of other equally damaging exotic species (Simberloff 2001). For example, after controlling invasive *Foeniculum vulgare* (Fennel) on Santa Cruz Island there was no increase in native species cover due to a secondary invasion of Mediterranean annual grasses (Odgen *et al.* 2005). The importance of determining what will replace the treated invasive plant species after control efforts was highlighted by Cronk and Fuller (2001), who also point out that there is no point in eradicating an invader if it is to be replaced by a worse one.

The interesting result from the experimental subplots was the difference between physical control treatments and the suite of species which followed control efforts which was not totally predicted by the seedbank experiment. A very small percentage (18.0%) of weeds established under frilled *Syzygium jambos* adults, and consisted mainly of one weedy species, *Oxalis corniculatus*, which proved easy to manually remove during subsequent weeding efforts (Chapter 4). While this species was observed as occurring in the seedbank trial, the prevalence of it alone in frilled plots was not predicted.

The suite of species found in cut plots was accurately accounted for in the seedbank experiment, with *Lantana camara*, *Eleusine indica* and *Sorghum sudanense* being the dominant weedy species found in cut plots after *Syzygium jambos* control efforts. *Oxalis corniculatus* was

absent from the majority of the cut plots. The weed incidence in cut plots was extremely high with an average cover value of 71.1%. These weeds also proved difficult to remove manually during subsequent weeding efforts (Chapter 4).

3.4.4 Native forest species regenerating after *Syzygium jambos* control efforts

The appearance of some of the native and introduced species commonly found in remnant native forest communities on Pitcairn were not accounted for during the seedbank experiment. This suggests that the native species which did appear after *Syzygium jambos* control efforts form part of a transient and not permanent seedbank. However, this was a much better result than that obtained from a study in Hawaiian dry forest where not a single naturally recruiting native seedling was found after two years (Cabin *et al.* 2002). While it was heartening to see some native and naturalised species emerge after *Syzygium jambos* control efforts, it was apparent that the species which emerged in plots were only those found adjacent to the treated areas.

A somewhat similar result was obtained by Ogden *et al.* (2005) who found after control treatment of *Foeniculum vulgare* (Fennel), the initial pilot experiments had suggested that native species would emerge after control efforts. However, this was not the result obtained during landscape level control efforts. It was subsequently realised that the trial experiments were only conducted in areas where native communities were adjacent to treated areas (Odgen *et al.* 2005).

Other interesting results were that many of the native fern species were found regenerating only in frilled subplots as most plots contained two subplots which were frilled and two which were cut. The native ferns tend to grow in the shade of the treated *Syzygium jambos* adults. This suggests that the frill *Syzygium jambos* treatment would suit the re-establishment of most of the native fern species rather than the cut treatment.

Pinus sp. (possibly *caribaea*) was found regenerating in 17.39% of cut subplots. While this is not very large percentage the fact that is capable of establishing in treated subplots is a cause for concern. In South Africa, Richardson (1997) noted that *Pinus* spp. predominately cultivated as forestry trees, coupled with alien species were found spilling into areas set aside for conservation purposes. Pines are now considered alien invaders throughout much of the Southern Hemisphere with reports of their invasive nature from New Zealand, Madagascar and Australia (Richardson & Higgins 1998; Cronk & Fuller 2001, Williams & Wardle 2005). More recently, studies throughout French Polynesia are discovering many *Pinus* spp. regenerating freely and these are now considered as invasive alien species there (J-Y Meyer *pers .comm*).

The aim of attempted control or eradication efforts for invasive species should be to establish the native vegetation as quickly as possible (Mack & Lonsdale 2000). In this case while there were some native species found regenerating in treated plots, they did not occur in sufficient

numbers or densities for the desired and necessary establishment of the native vegetation post *Syzygium jambos* control efforts.

3.4.5 Cost of *Syzygium jambos* control efforts

The cost of *Syzygium jambos* control is comparible to the published costs of control for other invasive species. For example; the cost of *Rhododendron ponticum* control in the UK is £526 per hectare (Dehnen-Schmutz *et al.* 2004). Some of the highest published costs for invasive species control are found on Islands. On Mauritius, control of invasive species costs US$3,000 per hectare (Rayment & White 2007) and on Laysan Island the costs of controlling *Cenchrus echinatus* (sandbur) was estimated at $2600 per hectare (Flint & Rehkemper 2000).

With *Syzygium jambos* control the highest costs are associated with start up costs for equipment and labour costs. *Syzygium jambos* control using the cut treatment and local labour is €18,939.88 per hectare and the frill treatment €11,826.31 per hectare. There are 17 hectares of *Syzygium jambos* forest on Pitcairn Island (Kingston & Waldren 2003) it forms the largest area of forest cover on the island. These high costs make *Syzygium jambos* control on Pitcairn Island an expensive invasive species to control. The only other comparable costs come from California with invasive species control there costing between US$600-US$75,000 per hectare (Rejmánek *et al.* 2000). If labour costs are excluded by using free/"volunteer" labour the cost of the cut treatment could be drastically reduced to €767.63 per hectare and the cost of the frill treatment could be reduced to €253.81.

The difference between *Syzygium jambos* control efforts and control efforts for other invasive species, is the high density in which the species occurs. Mean *Syzygium jambos* adult density on Pitcairn Island is $2.3m^{-2}$ adults compared to $0.5\text{-}0.7m^{-2}$ stems of *Syzygium jambos in* Puerto Rico (Brown *et al.* 2006). Other shrubby invasive species such as *Rhododendron ponticum* have seedling densities in the range of 9.9 m^{-2} (Dehnen-Schmutz *et al.* 2004). On Pitcairn Island the mean seedling density for *Syzygium jambos* is 56.1 m^{-2}. *Lonicera maackii,* another shrubby invasive species occurs also in lower densities with 2.1 plants recorded m^{-2} (Hartman & McCarthy 2004).

The serious nature of *Syzygium jambos* invasion with extremely high densities of *Syzygium jambos* with 100% cover in some forested areas of the island demonstrates the severe nature of the invasion. The species itself being an invasive tree instead of shrub or herbaceous grass also makes control efforts more physically demanding and time consuming and hence much more costly.

Large amounts of money are spent every year on managing invasive species, for example, *Lythrum salicaria* (Purple loosestrife) control costs US$45 million, and *Melaleuca quinquenervia* control costs US$3-6 million in the United States per annum, even with these large sums eradication has still not being achieved. Simberloff (2000) points out that a long-lasting eradication effort could be achieved for ten or twenty times the current annual cost and highlights the fact that

resource managers need to think big to achieve success. On Pitcairn the cost of *Syzygium jambos* control is high but the successes from the trial plot treatments are also high. Cost should not be a deterrent to the further expansion of control efforts for *Syzygium jambos* as it has proven to be susceptible to control, one of the most important of the six requirements for a successful eradication programme highlighted by Myers *et al.* (2000).

General conclusions

Both physical control methods (cut and frill) and both chemical treatments (Tordon and Roundup) were successful in controlling *Syzygium jambos* on Pitcairn Island. As *Syzygium jambos* is a woody tree species additional effort to treat resprouting individuals was needed over the treatment period. Few native species were found regenerating in the treated subplots which indicates that once treated a secondary invasion with weedy species was the likely replacement vegetation. Increases in the rat population over the duration of the project (*pers. obs.)* also are a cause for concern as they can further facilitate the spread of *Syzygium jambos*. While the control treatments are successful a long term (>5 years) monitoring programme is required to ensure that treated and controlled areas remain so. The cost of treating and controlling *Syzygium jambos* is minimal in terms of capital start up and chemical costs, though the treatments are labour intensive and thus expensive. One way of reducing labour costs in invasive species control efforts is to use volunteer labour, this has proven to be popular with other invasive species control efforts such as, *Rhododendron ponticum* control in Killarney, Ireland. The use of volunteer labour to control *Syzygium jambos* on Pitcairn would also provide a constant source of tourism revenue to the Island and Islanders.

"The next century will, I believe, be the era of restoration in ecology"
E.O. Wilson 1992

Chapter 4

Forest restoration on Pitcairn Island after *Syzygium jambos* control

4.1 Introduction

4.1.1 Forest restoration

The implicit goal of non-native species eradication programmes is to facilitate the restoration of historic community composition and or function (DiTomaso 2000; Manchester & Bullock 2000). Invasive species control and habitat restoration are now considered integrally linked (Fielder & Karieva 1998). The continued mounting pressure of invasive species on habitats requires the relationship between invasive species control and habitat restoration to become even more integrated and more innovative (Suding 2005).

Restoration of woodland ecosystems differs in scale and time from most other types of restoration (Ashby 1997). This is because trees form the dominant organisms in forests. Restoration of tree cover is usually the primary goal in woodland restoration because its aids repair of other facets of forest structure and function (Howell 1986; Stantruf *et al.* 2001).

The goals and methods of forest restoration differ from standard forestry practice. Reforestation is the restoration of any kind of tree cover many do not provide suitable habitat for plant species that once inhabited the forest ecosystem that the forestry plantation replaces (Elliott *et al.* 2006). Forest restoration is the attempted establishment of the original forest ecosystems, though in many instances re-establishing all the species that live in an original forest ecosystem cannot be replaced in a single step. The aim is to restore former levels of the forest ecosystem structure and as much function as possible (Elliott *et al.* 2006). Biodiversity in restored forest is required for wildlife conservation, environmental protection, eco-tourism and supply of forest products to local communities (Elliott *et al.* 2006). This attitude to forest restoration points out that restoration should where possible seek to integrate with local agricultural needs and landscape services, while improving and expanding the areas of available habitat for native species (Sayer *et al* 2004).

Several countries, especially those in the east and south Asian regions have embarked on major programs for the restoration of degraded forest ecosystems. Vietnam is looking for international support to reforest 5 million ha of uplands degraded by fire and in Thailand, as part of the Royal Jubilee Program in 1996, a 0.8 million hectare forest restoration programme for watershed protection was planned (Elliott *et al.* 2006). Smaller programs in Indonesia and the Philippines aim to reduce extensive areas of invasive *Imperata* grasslands and replace them with forest (Sayer *et al.* 2001).

Restoration on islands provides the opportunity to reduce the stochastic threats to rare species and raises opportunities to investigate how species interact (Towns & Ballantine 1993). A large scale forest restoration project at Aratiatia, North island, New Zealand, found after three decades of observations that the large scale planting of fast growing, short lived native shrubs and trees was successful in restoring woody vegetation to large areas of formerly bare ground, and in ecological terms the restored vegetation mimicked young secondary native forest (Smale *et al.* 2001).

4.1.2 The role of horticulture in forest restoration

The urgent need for basic research on how to propagate dry forest species was highlighted in a study in Hawaiian dry forest by Cabin *et al.* (2002) who found no naturally recruiting seedlings of some of the dry forest species under investigation. In Brazil the low numbers of native species available in nurseries at certain times is considered the greatest limitation to forest restoration, and it was highlighted that most projects use only 35 of the available 100 to 120 native tree species (de Souza & Batista 2004). Similar forest restoration practices in Mexican cloud forest also highlighted the fact that the use of native tree species for forest restoration has been constrained by lack of knowledge about their requirements for propagation, survival and growth (Alvarez-Aquino *et al.* 2004).

The choices of propagation methods for forest restoration include planting seed, planting container grown plants and bare root transplants (Elliott *et al.* 2006). There are advantages and disadvantages associated with the use of seeds or plant parts in a founding population (Guerrant 1996).Using native seed to plant or direct seed into sites has been used in many forest restoration projects for example, planted seedlings of native tree and shrub species in Ohio (Harman & McCarthy 2004), New Zealand (Lovegrove *et al.* 2000), Islands in Western Australia (Rippey *et al.* 2000), Hong Kong (Lai & Wong 2005) Mexico (Alvarez-Aquino *et al.* 2004),Uganda (Chapman *et al.* 1999) and Brazil (Zamith & Scarano 2006). The less popular method of direct seeding native trees has been used in Canada (Matthes *et al.* 2003) and Hawaii (Cabin *et al.* 2002). Wild collected seed is considered best for restoration purposes as it has a better chance of being genetically representative of healthy naturally occurring populations. Collecting seed is also considered less damaging to donor plants than collecting plant parts (Guerrant 1996).

Collecting plant parts and using cuttings to generate new plant populations has been underutilized as a way of generating species for restoration projects (Guerrant 1996). Taking cuttings from donor plants, however, may reduce their fitness or lower there survivorship either directly or indirectly by introducing plant pathogens. The great advantage of taking cuttings or other plant parts in an experimental setting are they are replicates of a genetically identical population (Guerrant 1996).

4.1.3 Measuring restoration success

Many restoration projects do not make any measure of restoration success because projects run in most instances for less than five years and having the financial resources to carry out assessments of restoration success are expensive. In a survey of 468 articles on restoration success after seeding or planting only 68 evaluated success or otherwise of the project (Ruiz-Jaen 2005).

The Society of Ecological Restoration International (SER) (2004) list nine ecosystem attributes as a guideline for measuring restoration success. The nine attributes they suggest that a restored ecosystem should have are: 1. similar diversity and community structure in comparison to reference sites, 2. have indigenous species, 3. contain functional groups necessary for long term stability, 4. the environment should be capable of sustaining reproducing populations., 5. normal functioning, 6. landscape integration, 7. elimination of potential threats, 8. resilience to natural disturbance and 9. self-sustainability. As most projects do not have the funds or time to investigate all these attributes more practical measures which incorporate and classify the SER categories that could be measured in some form are: 1. diversity or species richness 2. vegetation structure and 3. ecological process (Ruin-Jaen 2005). Elliott *et al.* (2006) highlights that the success of forest restoration should be measured in terms of the return of a multi-layered canopy, an increase in the numbers of native species especially rare and keystone species, and improved soil conditions.

4.1.4. Pitcairn Island Forest restoration

The objective of forest restoration on Pitcairn Island was to increase biodiversity and achieve a "native" forest type ecosystem on Pitcairn Island in place of the invasive *Syzygium jambos* forest. The composition of the seed bank (see Chapter 3; Section 3.4.2) was found to be mostly deficient in native plant species thus the regeneration potential of the sites where *Syzygium jambos* was treated, if left to their own devices, was negligible.

The decision on what species to use to achieve the goals of both habitat and species restoration had to be made. Rather than debate the functionality of species or decide which species were keystone and which not, we choose species that represented those found in the remnants of the woody native forest of Pitcairn Island, with their associated Polynesian plant introductions and more recent historical introductions of fruit trees as found occurring in native forest woodland types described by Kingston & Waldren (2003). Included in the planting mix were rare endemic species such as *Coprosma benefica* (Red berry), along with rare native species such as *Xylosma suaveolens* (Sharkwood), *Guettarda speciosa* (High White - Pitcairn's National Flower) and *Hibiscus australense* (Fatu) newly recorded for Pitcairn Island in 1997 (Kingston 2001) and species which provide material for carving curios *Calophyllum inophyllum* (Tamanu) and *Cordia subcordata* (Tau). These species are native to Polynesia and there are no reports of them invading in other pacific island ecosystems (Space 2002).

While the species chosen do not represent a natural forest assemblage on Pitcairn Island, a range of native species were used to provide vegetation cover and increase the biodiversity in plots where *Syzygium jambos* was controlled (Chapter 3). We felt it was important to restore a "quasi-natural" forest which can supply resources in terms of timber and fruits to the local community (Higgs 1997; Maunder *et al* 1998; Sayer *et al.* 2004). An approach that is a mix of the arcadian (Swart *et al.* 2001) and the focal-species approach (Lambeck 1997 & 1999; but see: Lindenmayer *et al.* 2002).

4.1.5 Aims of this study

The main aims of the investigations performed in this chapter were to propagate from seed, cuttings and plant parts the native and economically useful species found in native forest on Pitcairn Island and to plant the species propagated in plots where *Syzygium jambos* was controlled and record growth and survivorship for each species used. The specific aims were:

1. To propagate and cultivate as many as possible of the endemic and native species found in native forest on Pitcairn Island.
2. To use the plants grown to replace *Syzygium jambos* in treated plots (Chapter 3) and record their growth and survivorship.
3. To assess the success of attempted forest restoration in terms of species richness and soil processes.

4.2 Materials and methods

4.2.1 Sites selected for forest restoration on Pitcairn Island

All 21 *Syzygium jambos* experimental control plots (Section 2.2.2 and Table 2.3) each with 4, 10x10m^2 subplots (84 subplots in total) (Chapter 3, Section 3.2.2) were used as forest restoration sites. The plots contained the mix of physical treatments applied to *Syzygium jambos*, which were either cut or frilled.

4.2.2 Species selection for forest restoration on Pitcairn

The species outlined in Table 4.1 were selected for forest restoration efforts on Pitcairn Island. Trees and shrubs were selected over herbaceous members of the ground flora, which is composed mostly of fern species as it was felt these would arrive in time given their dispersal ability once the forest canopy structure was replaced.

Some species which were not found occurring in native forest types described by Kingston & Waldren (2003) were included in the propagation and planting mix as they are useful species to the islanders or are extremely rare on the Island.

4.2.3 Propagation and cultivation of selected species

A small nursery was constructed to propagate and grow native and economically useful species to replace *Syzygium jambos* in the trial plots (Table 4.1). All the plant material collected was from local forest sources and efforts were made to collect from a variety of individuals from different populations found throughout the island which is deemed the most suitable source for maintaining local ecotypes and having no adverse effects on the integrity of local gene pools (Knapp & Rice 1994; Linhart 1995; McKay *et al.* 2005). Selection of seed and cutting material was thus gathered from a variety of locations (where possible) to minimize "unconscious selection" as recommended by McKay *et al.* (2005).

Capillary beds were constructed with rough sawn pine timber, capillary matting (PinePac, New Zealand) and washed sand from the beach at Tedside. A shade cloth canopy was erected over the beds on timber poles. Propagation was carried out in frames constructed from treated plywood and covered with polythene (Plate 5: Pitcairn Island Nursery *est.* July 2003).

Table 4.1 Species selected for forest restoration on Pitcairn Island

Species selected for forest restoration	Local name	Growth Habit	Propagation Method
Alyxia scandens	Jay's bush	Shrub	Cuttings
Angiopteris chauliodonta	Nehe *	Fern	Stipule bases
Calophyllum inophyllum	Tamanu Δ	Tree	Stem cuttings
Cerbera manghas	Hulianda	Tree	Seed (drupe) & wild seedlings
Citrus aurantium	Orange Φ	Tree	Stem cuttings
Citrus aurantifolia	Lime Φ	Tree	Stem cuttings
Citrus medica	Thorny lime Φ	Tree	Stem cuttings
Citrus reticulata	Mandarin Φ	Tree	Stem cuttings
Coprosma benefica	Red berry *	Shrub	Stem cuttings & seed (berry)
Cordia subcordata	Tau Δ	Tree	Stem cuttings & seed (drupe)
Cyathea medullaris	Man fern	Fern	Sporlings & division
Cyclophyllum barbatum	Hard Jessamy	Shrub	Stem cuttings & seed (drupe)
Glochidion pitcairnense	Mahame	Tree	Stem cuttings & Seed (schizocarp)
Guettarda speciosa	High White	Tree	Stem cuttings & seed (drupe)
Hernandia sonora	Tonina nut	Tree	Trunk sprouts & seed (drupe)
Hibiscus australense	Fatu	Shrub	Stem cuttings
Hibiscus tiliaceus	Pulau	Tree	Stem poles, cuttings & seed (capsule)
Homalium taypau	Taypau *	Tree	Seed (capsule)
Jasminum didymum	Easter vine	Vine	Stem cuttings
Macadamia integrifolia	Macadamia nut Φ	Tree	Seed
Meterosideros collina	Rata	Tree	Stem cuttings, seed (capsule) & wild seedlings
Morinda citrifolia	Nanu Δ	Shrub	Stem cuttings & seed (fleshy syncarp)
Morinda myrtifolia	Running Jessamy	Vine	Stem cuttings
Pandanus tectorius	Thatch	Tree	Seed (syncarp)
Pisonia austro-orientalis	Wae-wae	Tree	Stem cuttings
Psydrax odorata	Jessamy	Shrub	Stem cuttings, seed (drupe) & wild seedlings
Thespesia populnea	Miro Δ	Tree	Stem cuttings & seed (capsule)
Xylosma suaveolens	Sharkwood	Tree	Stem cuttings, seed (berry) & wild seedlings

*** Endemic to Pitcairn Island**
Δ Polynesian Introduction (1220-1650)
Φ Historic fruit introduction (1800-1900's)
Highlighted in green are species not found occurring in native forest community types described by Kingston 2003 but were included in the planting mix as they are useful species to the islanders or are extremely rare on the Island.

Plate 5 Pitcairn Island Nursery with capillary beds at the foreground and propagation frames sealed with polythene towards the rear and covered in shade cloth. The photo on the right shows inside the propagation frames in Pitcairn Island nursery demonstrating the range of propagation methods used: cuttings (bottom) division (centre) and seed (top).

The propagation frames constructed in the nursery were a modified version of commonly used "cold frames" (which are traditionally placed outside on the ground in cold northern hemisphere climates). Each frame was raised 80cm from ground level, sealed with polythene, and a hinged lid was constructed for ease of access (Plate 5). The frames help to increase temperature, maintain humidity and allow light penetration, favourable conditions for root development and seedling germination (McMillan Browse 1992).

Various standard propagation methods were used to produce plants for forest restoration; cuttings, seed and division (McMillan Browse 1992).

Cuttings

Growing plants from cuttings is the most popular method of vegetative propagation. The main difficulty with stem cuttings is that a stem separated from its parent plant has to survive the loss of moisture through transpiration from its leaves and with no roots for uptake of moisture (McMillan Browse 1992).

The majority of trees and shrubs on Pitcairn are evergreen and cuttings were prepared using the standard method of taking evergreen cuttings (McMillan Browse 1992). The cuttings taken from Pitcairn species identified in Table 4.1 were 10-15cm in length depending on the length of available stem material. Efforts were made to collect actively growing vegetative shoots free from pest and diseases. Plastic diving bags which were dampened with water before collecting the material and sealed immediately after material was placed inside were used.

A secateurs and grafting knife were used in the preparation of cuttings, once prepared they were placed in commercial seed and potting compost (50% peat: 50% sand) in a 3½ inch (8.5cm) plastic pot and the pot was placed directly into the plastic frame (Figure 3.2). This method was successful for the many of trees and shrubs selected for restoration efforts (Table 4.1). Callus tissue developed within three weeks on most species and rooting occurred after 1½ to 3 months.

Once the cuttings were rooted, each individual was potted on into individual 8.5cm pots using locally derived potting compost[8](which is a version of the standard John Innes No. 1 Compost[9]) and placed under shadecloth on the capillary beds in the nursery, watered regularly and potted into larger sized pots as growth required.

Seed

Growing plants from seed is the most prolific method of plant production and minimizes damage to the parent plant. There is an enormous variation in seed sizes. Small dust like seed such as *Meterosideros collina* are known to have low germination and survival rates, where as larger seeds like *Cerbera manghas* and *Hernandia sonora* are produced in smaller numbers but germinate and establish well (McMillan Browse 1992). All fruits (berries, capsules, drupes, schizocarps) were collected when ripe and the seed extracted. Seeds were collected and stored in brown paper manila envelopes and extracted and cleaned before sowing.

Seed extraction for schizocarps and capsules is relatively easy as the seed fall out when the capsule or schizocarp splits. Species on Pitcairn with capsules and schizocarps are generally evolved for wind dispersal – amenochory (Kingston 2001). Seed extraction for berry and drupes involves removing the fleshy pulp from around the seed by squashing and cutting followed by hand cleaning, picking, washing and decanting (McMillan Browse 1992). Species on Pitcairn with berries and drupes are evolved for animal dispersal-zoochory (Kingston 2001).

Once extracted seed were placed on a seed and potting compost mix (50% peat: 50% sand) in 8.5cm plastic pots and covered with sieved compost to the same depth as the seed themselves, and placed in the plastic frame to germinate. Most seed germinated within 3 months and upon development of "true leaves" (*i.e.* leaves formed after the cotyledons) were potted up into larger pots using the locally devised compost mix [1] and placed on the capillary beds under shadecloth to grow on.

Collection of Wildlings

On Pitcairn many berried plants for example, *Coprosma benefica* and *Xylosma suaveolens* produce seedlings in the immediate vicinity of adult plants, demonstrating the lack of a dispersal agent on the island (Kingston & Waldren 2005); these seedlings were also collected and grown on in the island nursery for planting in experimental plots. *Meterosideros collina* colonises bare scarred surfaces along roadsides and many seedlings germinated in these conditions, similar to other *Meterosideros* species such as *Meterosideros excelsa* which colonises landslide scars on Korapuki Island, New Zealand (Towns 2002). *Meterosideros collina* seedlings were collected before road grading works and grown on in the nursery.

---

[8] Potting compost: 2 parts seed and potting compost, 5 parts local loam with stone and root removed, 1 part fine quarry gravel and 40 grams of Ammonium Nitrate fertiliser (only fertiliser available on the island)

[9] John Innes No. 1 Potting Compost: 7 parts loam; 3 parts peat; 3 parts sand, ¾ oz. ground limestone and 4 oz of any fertiliser base.

Division

Dividing is a common method of propagation for many herbaceous plants and ferns. The best time to divide is when new vegetative shoots are being produced. Along the road sides of Pitcairn Island many native ferns produce sporelings and plants which can easily be collected and divided for propagation purposes for example, *Cyathea medullaris*. The plants were dug up and divided with a knife ensuring a rosette of leaves accompanied every rooted piece and the separated pieces potted into appropriately sized pots using locally devised compost [1] and grown on under shadecloth in the nursery.

Stipule bases

An unusual method of vegetative propagation exits for members of the fern family Marattiaceae of which the endemic fern *Angiopteris chauliodonta* is a member. Large fleshy globular "stipules" found at the base of the fronds can be removed from parent plants causing minimum damage. In many cases large stipules detach from the parent plants when mature. These stipules when buried in compost, or planted, sprout new fronds. A nursery stock collection of *Angiopteris chauliodonta* was developed for restocking sites as recommended by Kingston & Waldren (2002) and Kingston *et al.* (2004).

4.2.4 Permanent plot planting

Twelve plots consisting of 48 subplots were planted during March-May 2004 and a further eight plots with 32 subplots planted during March and April 2005. Plot 17 at Trevor's Canyon, which consisted of 4 subplots, was planted as a fruit orchard using the historical fruit tree varieties found on the island. The planting in this orchard plot consisted of 43 *Citrus reticulata* (mandarin); 55 *Citrus aurantifolia* and 30 *Citrus medica* (limes) and 106 *Citrus aurantium* (orange) trees all of which were propagated in the island nursery. In other sites fruit species were only used in plots on flat ground and in plots accessible from the road.

Seventy plants were planted in each plot consisting of two subplots with high density planting (20 plants per subplot) and two subplots with low density planting (15 plants per subplot) (see plot layout Chapter 3; Figure 3.0); though in hindsight a more contrasting species number would have been better. A total of 1400 plants were used to plant all the sites, all of which were propagated from local material grown in the island nursery.

The ideal ratio for forest restoration planting is 40:60; small trees, shrubs & fern: large trees were applied to plots where possible, which allows for a good shrub matrix within the wood (Gilbert & Anderson 1998). However, in many cases the ratio was 50:50 or less.

Several individuals of a given species were planted in each plot when numbers were available to do so and one at each site when numbers were low. Plants were placed randomly within each subplot with taller tree species scattered among the smaller tree, shrub and fern species. Planting was carried out using an adze and one pot 8.5cm of local compost mix was added to each planting hole. Plants were arranged outside the nursery shadecloth in bright sunlight at least one

week before planting and watered thoroughly before leaving the nursery, no additional water was supplied once planted.

4.2.5 Mortality, height and basal diameter measurements

Plant height (cm) was recorded with a measuring tape from the soil surface to the top of the stem or fern frond, and plant diameter (mm) was recorded using callipers at the base of the stem at soil level immediately after planting. A reference map marking the location of each plant was prepared for each plot (Appendix CD). Replacement planting was carried out in two plots(Graveyard 1 & 2) two weeks after planting as heavy rain washed out the newly planted plants. No further replacement plantings were carried out before the end of the experiment in February 2006. After the first period of ten months, May 2004 – March 2005; height was again measured. The remaining 8 plots were planted during May 2005 were re-measured after a period of ten months in March 2006 by the project manager. The Trevor's Canyon plot (17), planted with *Citrus* varieties was not re-measured. Height and basal diameter growth was calculated by subtracting the final measurement from the initial measurement after each period of ten months (Equation 4.1).

$$\textbf{Growth} = \textbf{Mf} - \textbf{Mi}$$

Mf = final measurement & **Mi** = initial measurement

Equation 4.1

Odds ratios for survival of plants in the different treatments were calculated (Sokal and Rohlf 1995). An odds ratio (ψ) compares the likelihood of an outcome in one treatment relative to a second treatment. The proportional responses for example can be the percentage of live plants: for example, response such as survival is ψ times more likely is more likely under treatment i (for example frill treatment) than under j (cut treatment) (Equation 4.2)

$$\Psi = \frac{pi/qi}{pj/qj}$$

where p and q are proportional responses to the treatment i and j

Equation 4.2

4.2.6 Native tree direct seed experiment

During initial plot planting in March 2004–May 2004 (48 subplots: 12 plots) an experiment was conducted to determine if adequate native tree regeneration could be achieved by broadcasting tree seed into treated sites. Freshly ripened seed were collected from local sources of the tree species found in native forest: *Meterosideros collina* seed were collected and mixed from 20 individual trees along Big Ridge, *Hibiscus tiliaceus* seed were collected and mixed from 20 native trees at Pulau and *Homalium taypau* seed was collected from the only individual that was seen to produce seed at The Palm. One hundred seed of *Hibiscus tiliaceus*, 200 seed of *Meterosideros collina* and 10 seed of *Homalium taypau* were scattered by hand into 2 x 2 m^2 marked grids in the centre of the 12 treated sites. The 2x2m^2 grids were searched for seedlings during plot surveying and measuring from March 2005.

4.2.7 Permanent plot weeding treatment

All plots including the planted [12] and unplanted [8] were not weeded during the period from May 2004 to March 2005. The eight remaining unplanted plots in 2004 were the subsequently planted in May 2005 and weeded once a month for a period of ten months until March 2006. A summary of all the factors set in the experimental treatment were:

- *Syzygium jambos* physical treatment: 2 levels (cut and frill)
- Planting density: 2 levels (High and Low)
- Plot maintenance: 2 levels (weeded and not weeded).

4.2.8 Data handling and analysis

Data matrices were compiled using MS EXCEL. Calculations and graphs were also performed in MS EXCEL and standard errors calculated and manually assigned to the graphs. Analysis of variance tests, ANOVA and tests for normality (Cochran's test) were carried out in GMAV5 (Underwood *et al.* 2004).

4.3 Results

4.3.1 Plant survival

Fourteen hundred plants were planted in 20 experimental plots (70 per plot) (Table 2.3) using 28 different species of which eight hundred and thirty-three survived during the experimental period (March 2004 – February 2006), giving a mean percentage survival of 59.5(± 19.1)% across all sites and treatments. The highest percentage plant survival (87.14%) was found in plot 19 (Tonina Valley) and the lowest (21.43%) at plot 5 (Lower Mema) (Figure 4.3). All the sites located near Tedside; plot 4, 5, and 6 (Tedside to the Sea, Lower Mema and Upper Mema), were browsed severely by goats during 2005. Other plots with low plant survival percentages were plots 8 & 9 (Corner at the Hollow & Devil's Elbow) (Figure 4.1) in these plots *Syzygium jambos* was cut from all the plots (cut treatment).

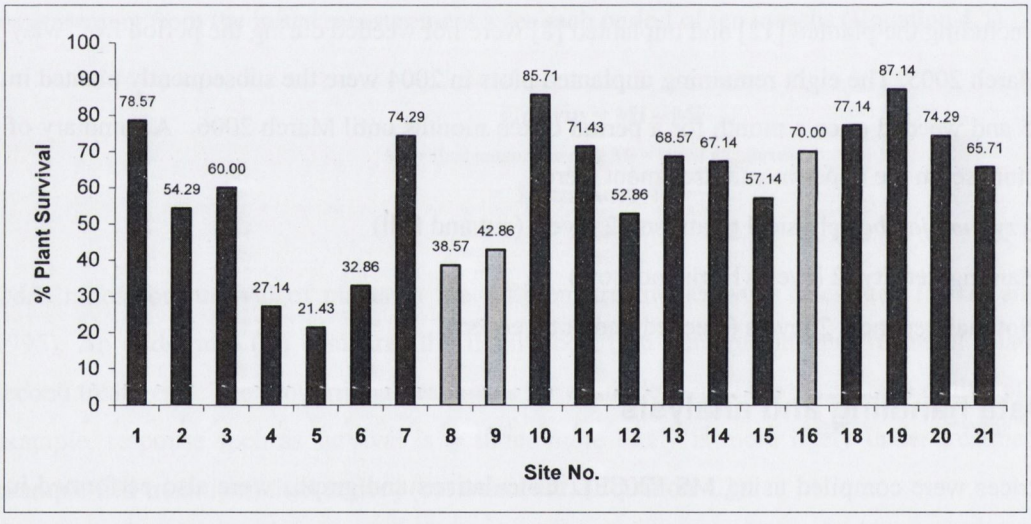


Figure 4.1 Percentage plant survival for each of the twenty plots chosen for experimental purposes. Sites marked in black bars hold two subplots with *Syzygium jambos* cut treatment and two subplots with frill treatment. Plots marked only in grey contain four subplots with *Syzygium jambos* cut treatment.

Plots 4, 5 & 6 which were severely browsed were not used for statistical analysis purposes leaving 17 plots available for statistical analysis and in order to balance for the experimental factors, five subplots from each treatment were chosen randomly from the remaining available 68 subplots. From the plots chosen for analysis, plant survival was highest in frilled subplots with high density planting and weeded (80.00 ± 14.57%) and lowest in cut subplots with high density planting and unweeded (42.00 ±10.36%) (Table 4.2). Planting density appeared a less important factor in plant survival than the weeding and *Syzygium jambos* treatment factors (Table 4.2).

Table 4.2: Mean percent survival of plants (±SD) in cut/frill; high density/low density and weeded/unweeded treatments in 40 experimental subplots with sample number (n=5).

		Syzygium jambos Cut		*Syzygium jambos* Frill	
		High Density (20)	**Low density (15)**	**High Density (20)**	**Low density (15)**
% Plant survival	**Weeded**	73.00±12.54	74.66±8.69	80.00±14.57	64.00±12.11
	Unweeded	42± 10.36	44.00±2.66	65±14.57	74.66± 1.33

Odds ratios were calculated for each treatment to compare the likelihood of plant survival under the different treatments (Table 4.3) each factor was controlled for the two other factors in calculating the ratio. Percentage plant survival was 8.39 times more likely under the frill *Syzygium jambos* treatment than the cut treatment which had a survival odds ratio of 2.99. Plant survival odds ratios were similar between high density planting and low density planting (4.71 & 4.20 respectively). Weeding treatment also had an effect on the odds ratio of percentage plant survival, with plants more likely to survive (10.24) in weeded treatments than unweeded treatments (2.56).

Table 4.3 Odds ratios for plant survival with different plot treatments. Each treatment Cut vs frill; High density vs. low density & weeded vs unweeded was controlled for the other two treatments in calculating the ratio

Syzygium jambos **treatment**	**Odds Ratio ψ**	**Planting density**	**Odds Ratio ψ**	**Weeding treatment**	**Odds Ratio ψ**
Cut	2.99	**High Density**	4.71	**Weed**	10.24
Frill	8.39	**Low Density**	4.20	**Unweeded**	2.56

A Cochran's test of heterogeneity of variance was performed and the data were found to be of equal variance (C=0.2221, not significant) and the data were analysed using analysis of variance. The analysis of variance on percentage plant survival found that *Syzygium jambos* treatment (p=0.001), weeding (p= 0.0001) and the interaction between *Syzygium jambos* treatment and weeding (p= 0.0002) were significant factors influencing plant survival (Table 4.4).

Table 4.4 ANOVA table for percentage plant survival in subplots with *Syzygium jambos* treatment; planting density and weeding treatment as significant factors

Source	*SS*	*DF*	*MS*	*F*	*P*
Treatment (T)	1562.500	1	1562.500	13.07	0.0010 **
Plant Density(D)	4.444	1	4.444	0.04	0.8483
Weeding (W)	2722.500	1	2722.500	22.77	0.0001 **
T X D	62.500	1	62.500	0.52	0.4750
T X W	2054.444	1	2054.444	17.18	0.0002 **
D x W	422.500	1	422.500	3.53	0.0693
TxDxW	401.111	1	401.111	3.35	0.0764
Residual	3826.666	32	119.583		
Total	11056.666	39			

** indicates significance at $p<0.001$

In order, to account for any issue of non-independence of treatments on percentage plant survival in sites a second analysis of variance was performed using one subplot from each site for each factor and similar results were obtained[10].

Percentage plant mortality across all subplots (80) was 40.5% (567 plants). The most common cause of mortality was drought 57% (326 plants), followed by goat browsing 37% (207 plants), falling debris 3% (17 plants, where *Syzygium jambos* which was frill treated and had started to disintegrate,) and washout 3% (17 plants) (Figure 4.2)

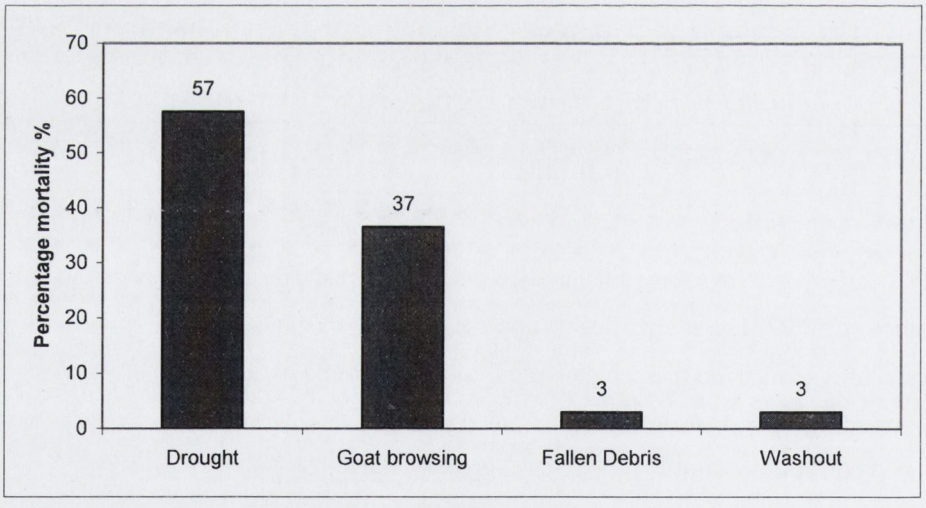


Figure 4.2 The causes of and percentage mortality of plants planted in all experimental subplots

4.3.2 Species Survival

Twenty-eight different species were used in the planting mix (Table 4.1), eighteen of which were planted in the majority of sites and plots. The species not planted across all subplots and treatments with their percentage survival in brackets were: *Angiopteris chauliodonta* (100%) an endemic fern

[10] Independent analysis of variance results: *Syzygium jambos* treatment ($F_{[1, 15]}$ 20.58; p= 0.0019); weeding ($F_{[1, 15]}$ 10.67; p= 0.0114) and treatment x density interaction ($F_{[1, 15]}$ 9.51; p= 0.0150) showing significant effects on percentage plant survival in plots. (Cochran's Test of data normality not significant C=0.5267).

with specialised habitat requirements (Kingston *et al.* 2004) which was only planted at one subplot which matched its habitat preference, plot 18: Brown's Water. *Alyxia scandens* (0%), an extremely rare shrub from which only a small amount of cutting material was obtained was planted in one subplot, plot 6: Upper Mema which was subsequently severely browsed by goats. *Eugenia reinwardtiana* (0%), another rare shrub on the island which is suited to coastal locations was only planted only at plot 2, Pulau. *Morinda myrtifolia* (38.46%), a rare climber was planted at five plots and the fruit and nut species of use to the islanders; *Citrus aurantium* (90.9%), *Citrus aurantifolia* (100%), *Citrus medica* (100%), *Citrus reticulata* (80%), *Morinda citrifolia* (100%) and *Macadamia integrifolia* (66.66%) were only planted in Flatland subplots.

Two of the native forest tree species *Homalium taypau* (61.66%) and *Meterosideros collina* (63.39%) were only planted in subplots which were weeded as no success was achieved with propagation efforts during 2003-2004, thus none were available for planting in subplots which were experimentally left unweeded during May 2004- March 2005 (Figure 4.3). Species survival varied significantly among the species planted (χ^2=224.2, d.f = 17, $p < 0.0001$), with mean survival for the eighteen widely planted species above 50% across all treatments (Table 4.5).

Species survival also varied with experimental treatment. Species survival in the cut and weed treatment were significantly different from each other (χ^2=281.6, d.f=17, p=<0.0001), as were species survival in the cut and unweeded treatment (χ^2=325.5, 15df, p=<0.0001). Species in the frill and weed treatment (χ^2=192.6, d.f=17, p<0.0001) and frill and unweeded treatment (χ^2=454, d.f=15, p<0.0001) also had significantly different survival percentage.

The species with the highest percentage survival in the cut and weed treatment were *Calophyllum inophyllum, Coprosma benefica, Cordia subcordata, Jasminum didymum and Pisonia austro-orientalis (*100%) these species are light loving species (Figure 4.3). The species which fared badly in the cut and weed treatment were *Homalium taypau* and *Cyathea medullaris* (50%), which are more shade loving species (Figure 4.3). The species with highest percentage survival in the cut and unweeded treatment was *Cerbera manghas* (90.47%) which demonstrates its ability to deal with both high light levels and a competitive environment (Figure 4.3). Table 4.5 ranks the species used in descending order of survival in treated plots.

Table 4.5 Species survival percentage in descending order in treated *Syzygium jambos* plots

Species	Percentage survival
Cerbera manghas	93.45%
Coprosma benefica	92.00%
Pandanus tectorius	88.78%
Calophyllum inophyllum	85.62%
Thespesia populnea	83.09%
Xylosma suaveolens	82.26%
Hibiscus australense	79.99%
Glochidion pitcairnense	79.88%
Jasminum didymum	79.04%
Cordia subcordata	75.01%
Psydrax odorata	73.39%
Hernandia sonora	73.52%
Guettarda speciosa	68.74%
Meterosideros collina	63.39%
Homalium taypau	61.66%
Cyathea medullaris	53.84%
Pisonia austro-orientalis	52.85%
Hibiscus tiliaceus	51.52%

Cyathea medullaris (15.38%) on the other hand seemed intolerant of high light and competitive environment in the cut and unweeded treatment. The cut and unweeded treatment also had the lowest mean species survival rate (58.56%) of all experimental treatments applied (Figure 4.6 & Table 4.6). *Cerbera manghas* (100%), *Coprosma benefica* (100%) and *Psydrax odorata* (100%) showed high percentage survival rates in the frill and weeded treatment as did the other fifteen species, this treatment had with the highest mean species survival rates of all the treatments (87.64%) (Figure 4.3 and Table 4.6). Within the frill and unweeded treatment, *Cerbera manghas* had 100% survival which once again demonstrated its ability to grow in a wide range of conditions. *Jasminum didymum* also with 100% survival also thrived showing its ability to deal with both light and shady conditions. *Pisonia austro-orientalis* (0%) did not tolerate the shady and competitive environment in the frill and unweeded treatment (Figure 4.3).

Table 4.6 Mean Percentage survival rates of 18 widely planted species with different experimental treatments

% Species survival				
	Cut & Weed	**Cut & Unweeded**	**Frill & Weed**	**Frill & Unweeded**
Mean	82.6	58.56	87.64	68.81
Standard Deviation	16.55	24.45	11.32	30.25
Standard Error	4.0	5.76	2.66	7.13

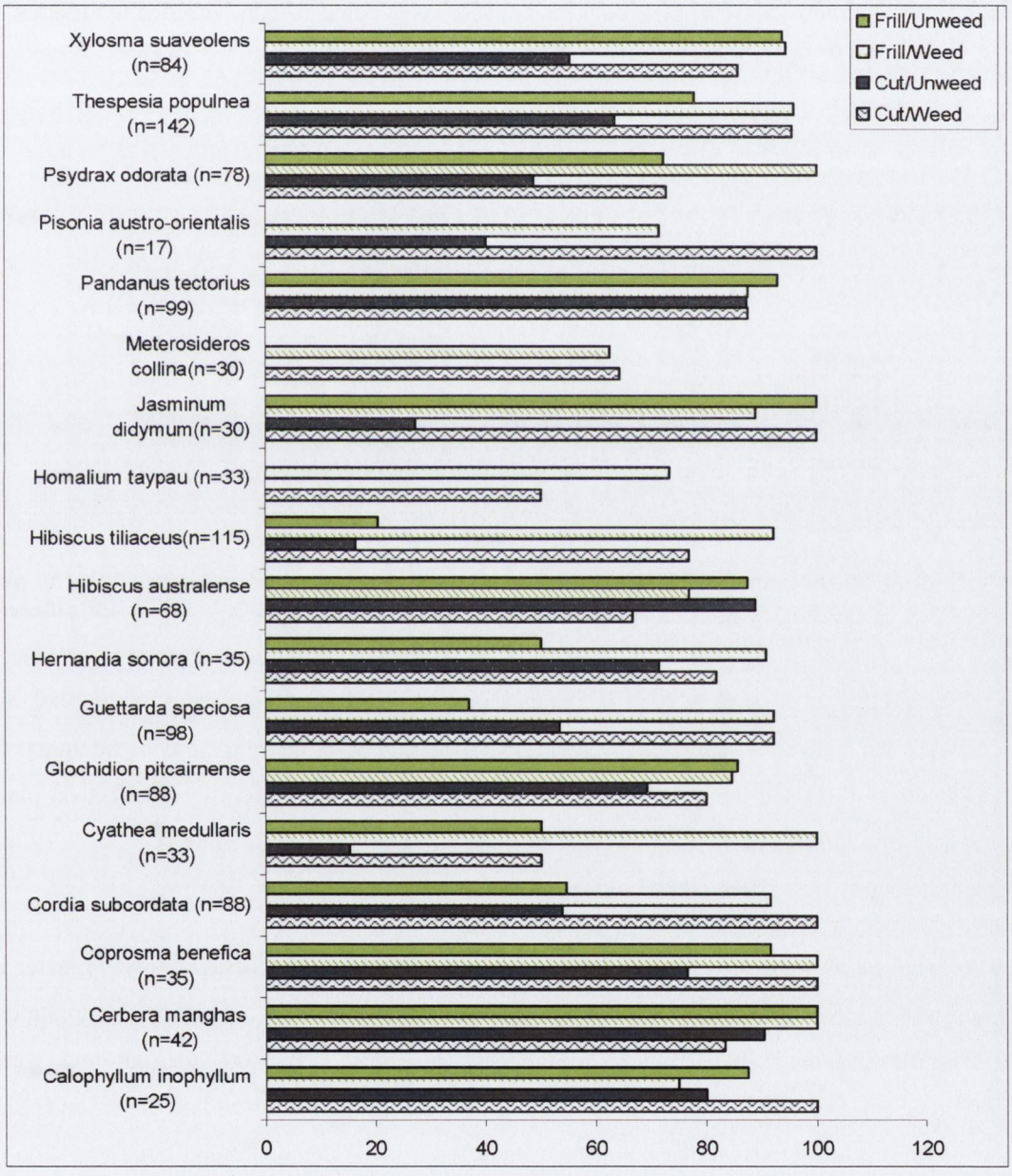


Figure 4.3 Selected widely planted species survival percentage with the significant experimental treatments

4.3.3 Plant Growth

Growth increment in plant height and basal diameter over the ten month experimental period differed with the experimental treatments, which were *Syzygium jambos* physical control: cut versus frill, and weeding regime: weeded versus unweeded. Highest plant height growth (cm) was recorded from the cut and unweeded treatment (70.82±11.90cm) and lowest from the frill and weeded treatment (17.91± 6.29cm) (Figure 4.4). The competition from weed species appears an important factor in plant growth as plants in the frill and unweeded treatment (43.86±9.54cm) also showed greater height growth (cm) than both the cut and weeded and frill and weeded treatments.

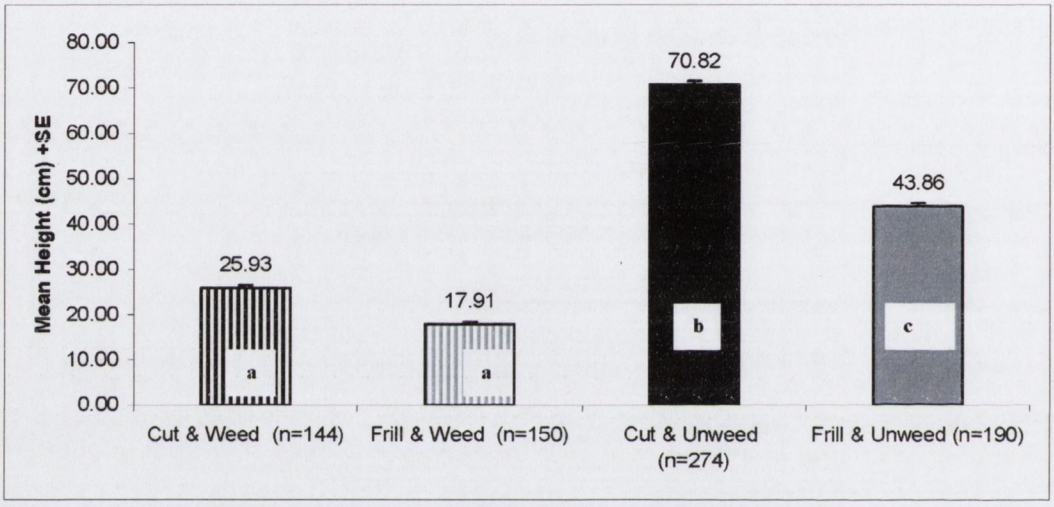


Figure 4.4 Mean height growth across the eighteen widely planted species in the different plot treatments: Cut & Weed, Cut & Unweeded, Frill & Weed, Frill & Unweeded: Bars with different letters are significantly different according to SNK post hoc test ($p<0.05$)

One hundred and thirty values from each treatment were used, and log transformed (C= 0.2980, ns) before analysis. An analysis of variance (ANOVA) showed that both *Syzygium jambos* treatment ($F_{[1, 519]}$ 15.82; p= 0.0001) and weeding ($F_{[1, 519]}$ 14.45; p= 0.0002) had a significant effect on plant height growth. The interaction between *Syzygium jambos* treatment and weeding ($F_{[1, 519]}$ 1.29; p= 0.2558) had no significant effect on plant height growth.

Average basal diameter growth of the eighteen widely planted species also was greater in the cut and unweeded experimental treatment (11.90± 11.98mm) and frill and unweeded treatment (7.38±9.54mm). Similar basal diameter growth was recorded from both the cut and weed (4.85±7.01mm) and frill and weed (4.59±6.29mm) plot treatments though standard deviation (±SD) from the average values were often greater than the average value demonstrating large variation in basal diameter growth (Figure 4.5).

An analysis of variance was performed using basal diameter measurements from each treatment which were log transformed to reduce heterogeneity of variances (C=0.3117,ns). The result found that both treatment ($F_{[1, 519]}$9.85;p=0.0018), weeding ($F_{[1, 519]}$37.01; p<0.0001) and the

interaction between treatment and weeding ($F_{[1, 519]}$ 6.04; p=0.0143) significantly influenced basal diameter growth.

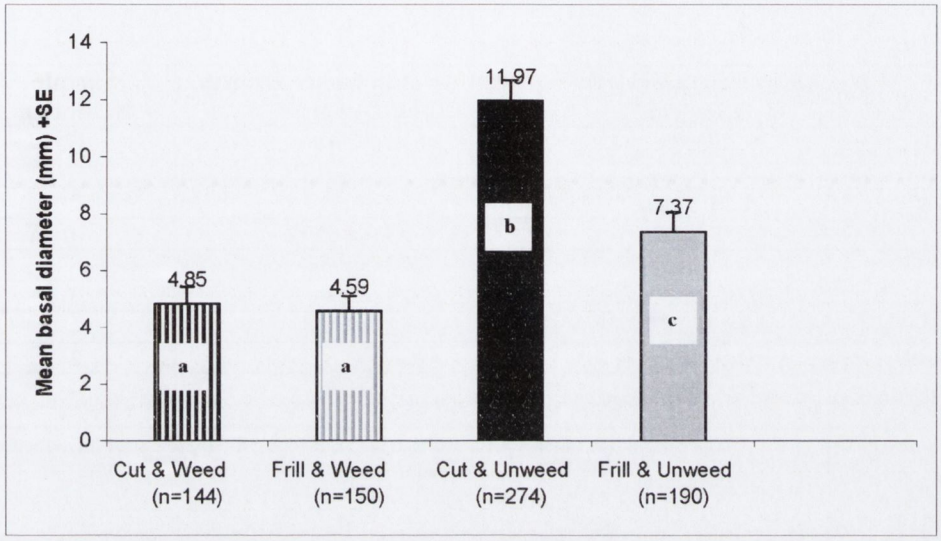


Figure 4.5 Mean basal diameter growth across the eighteen widely planted species in the different plot treatments: Cut & Weed, Cut & Unweeded, Frill & Weed, Frill & Unweeded: Bars with different letters are significantly different according to SNK post hoc test (p<0.05)

4.3.4 Species growth

The species of conservation or economic importance which were planted into few plots, such as the endemic fern species *Angiopteris chauliodonta* planted at plot 18 Browns Water, survived and produced both height and basal growth during the experiment (Table 4.7). The rare native climber *Morinda myrtifolia* showed negative growth with plot conditions unfavourable (Table 4.7). *Citrus aurantium*, *C. aurantifolia*, *C. medica* and *C. reticulata* also showed strong positive growth rates (Table 4.6), along with the economic nut species *Macadamia integrifolia*. *Morinda citrifolia*, another fruit of economic importance, had mean negative growth (Table 4.7). These results indicate that sites where *Syzygium jambos* has been treated are somewhat suitable for growing species of both economic and conservation importance.

Of the species widely planted across all plots and treatments, the species which demonstrated the highest height growth was *Hibiscus australense* with a mean growth of 231.46 (±144.33) cm. *Hibiscus australense* was first recorded on Pitcairn in 1997 and was only found in two locations on the island. It was reported then as growing to 3m (Kingston 2001), In ten months, the planted cuttings of this species are attaining heights of over two metres (Figure 4.11). Another species which grew very well across all treatments and sites was the endemic species *Coprosma benefica* with average height growth of 58.94(±35.9)cm during the ten months Only twelve individuals of this species remained on Pitcairn in 1997 (see also Chapter 5) (Kingston 2001) (Figure 4.11).

Table 4.7 Planted species height and basal diameter growth means (±SD) in experimental treated plots. Sample number (n) is the number of plants which survived and were measured to produce results shown.

Species	Mean Height Growth (±SD) cm	Mean Basal Diameter Growth (±SD)mm	Sample Number n
*Angiopteris chauliodonta**	9.1 (±15.6)	3.9(±3.4)	12
Morinda myrtifolia¤	-4.5 (±2.12)	0	2
Citrus aurantium Φ	24.5 (±7.7)	4.3 (±1.3)	10
Citrus aurantifolia Φ	36.1 (±32.4)	8.2 (±5.0)	8
Citrus medica Φ	60.0 (±21.2)	6.3 (±4.1)	3
Citrus reticulata Φ	42.2 (±22.2)	4.2 (±2.0)	4
Morinda citrifolia Δ	-7.5 (±6.3)	6.0 (±2.8)	2
Macadamia integrifolia Φ	20.0 (±22.9)	8.5 (±3.0)	4

*** Endemic to Pitcairn Island; ¤ Native to Pitcairn Island; Δ Polynesian Introduction (1220-1650); Φ Historic fruit introduction (1800-1900's)**

The mean native species height growth rates (±SD) over the experimental ten month period in all treatments in descending order are: *Hibiscus australense* (231.5±144.3cm), *Coprosma benefica* (58.9±35.5cm), *Cerbera manghas* (58.8±38.8cm), *Hibiscus tiliaceus* (47.8±36.9cm), *Xylosma suaveolens* (44.2±32.1cm), *Glochidion pitcairnense* (42.5±33.6cm), *Jasminum didymum* (34.5±79.7cm), *Pandanus tectorius* (29.5±48.5cm), *Cyathea medullaris* (24.7±24.7cm), *Homalium taypau* (22.0±24.4cm), *Hernandia sonora* (13.9±18.4cm), *Pisonia austro-orientalis* (9.9±13.0cm), *Guettarda speciosa* (9.1±9.7cm), *Meterosideros collina* (6.9±8.3cm) and *Psydrax odorata* (5.4±12.9cm). Of the Polynesian introduced species grown in plots mean height growth in descending order was as follows: *Thespesia populnea* (28.9±42.5cm) a tree species commonly used for carving curios, *Calophyllum inophyllum* (22.8±26.4cm) and *Cordia subcordata* (16.7±21.1cm) also used for carving on the island (Figure 4.6).

Mean height growth of individual species differed within the different experimental treatments. Height growth was greatest in the cut and unweeded treatment for *Calophyllum inophyllum*, *Coprosma benefica*, *Cordia subcordata*, *Cyathea medullaris*, *Glochidion pitcairnense*, *Hernandia sonora*, *Hibiscus australense*, *Hibiscus tiliaceus*, *Pandanus tectorius*, *Thespesia populnea* and *Xylosma suaveolens* (Figure 4.6). However, *Jasminum didymum*, *Pisonia austro-orientalis* and *Psydrax odorata* had greater height growth in the frill and unweeded treatment and *Cerbera manghas* grew best in frilled weeded plots, these species appear to prefer the more shaded conditions within the frill treatment for height growth.

Psydrax odorata also showed negative growth in the cut and unweeded treatment indicating a low tolerance to the high light conditions for growth. *Pandanus tectorius* on the other hand showed negative growth in the frill treatments, both weeded and unweeded, indicating its growth preference for situations with high light (Figure 4.6). *Meterosideros collina* and *Homalium taypau*

did not receive the unweeded treatment and their height growth rates were higher in the cut and weed treatment.

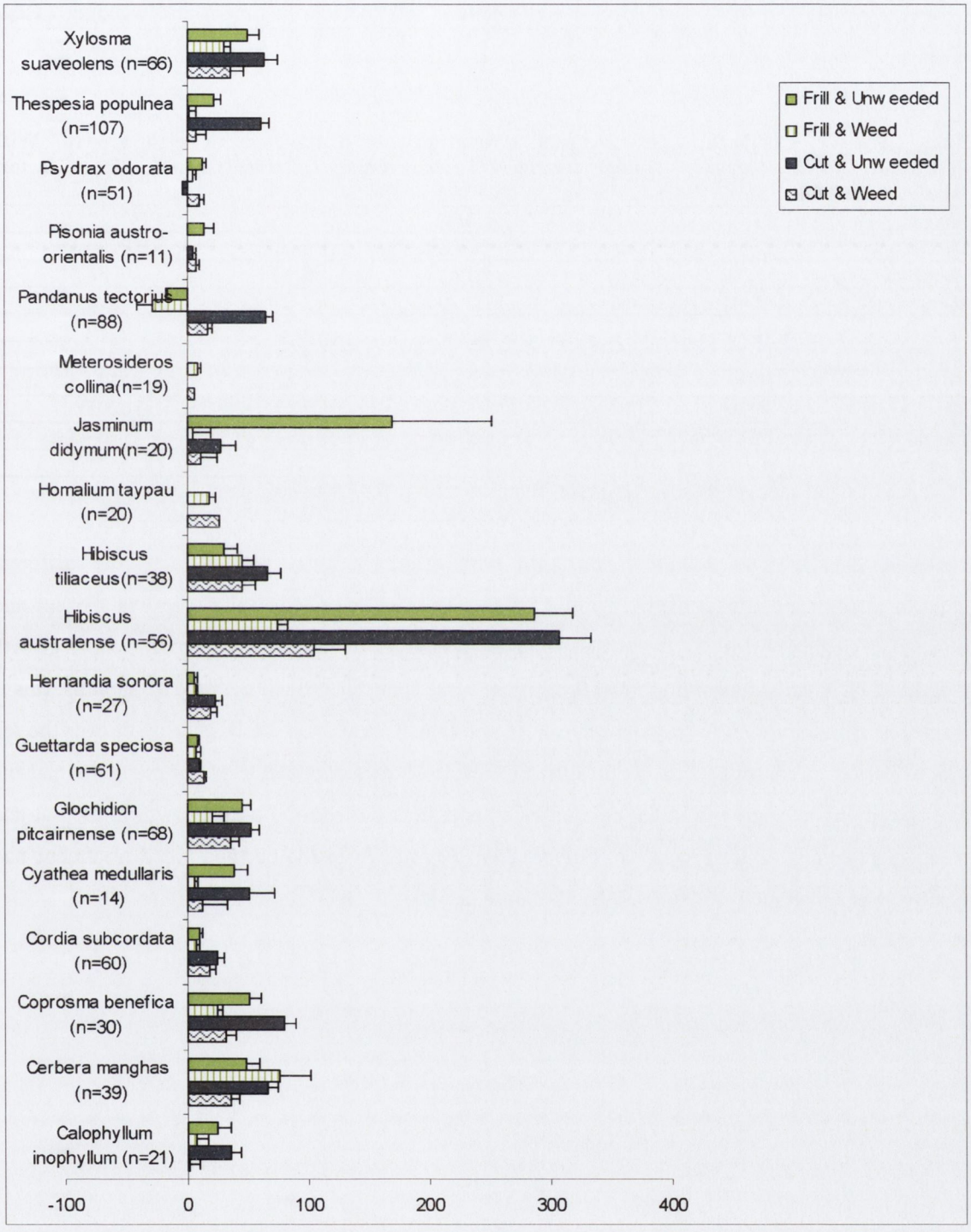


Figure 4.6 Mean individual species height growth (+SE) across all significant experimental treatments.

An analysis of variance was carried out using fourteen species excluding *Cyathea medullaris*, *Homalium Taypau*, *Meterosideros collina* and *Pisonia austro-orientalis* because they did not exist in all treatments. The data were squared to obtain positive values and log transformed in order to satisfy the criteria for using analysis of variance tests (Cochran's C=0.1370, ns). Mean height

growth was found to vary significantly between the fourteen species analysed, with *Syzygium jambos* treatment and weeding treatment. Significant interactions were found influencing height, between species and treatment, species and weeding and species, treatment and weeding (Table 4.8).

Table 4.8 ANOVA table for significance tests of treatments on planted species height growth. With individual species; *Syzygium jambos* treatment; and weeding treatment as significant factors influencing height

Source	*SS*	*DF*	*MS*	*F*	*P*
Species (Sp)	492.46	13	37.88	17.22	0.0000 **
Treatment (Tr)	19.73	1	19.73	8.97	0.0034 **
Weeding (We)	32.00	1	32.00	14.55	0.0002 **
Sp x Tr	67.42	13	5.18	2.36	0.0080 **
Sp x We	82.37	13	6.33	2.88	0.0013 **
Tr x We	2.68	1	2.68	1.22	0.2720 ns
Sp x Tr x We	56.39	13	4.33	1.97	0.0295 *
Residual	246.39	112	2.19		
Total	999.48	167			

** indicates significance at $p<0.01$; * indicates significance at $p<0.05$ & ns=not significant

Mean basal diameter growth varied both within and between species with the different experimental treatments applied (Figure 4.7). Basal diameter growth was highest in the cut and unweeded treatment for all the widely planted species showing similar results height growth (Figure 4.7). *Cyathea medullaris* had the largest basal diameter growth over the 10 months, this is a species of tree fern. *Hibiscus australense* did not demonstrate dramatic basal growth over the ten month period in contrast to its height growth. *Pandanus tectorius* and *Cerbera manghas* had the least basal diameter growth in the cut and weed treatment but showed most growth in the cut and weeded treatment. All species bar *Calophyllum inophyllum* in the cut and weed treatment had positive basal diameter growth over the 10 month period.

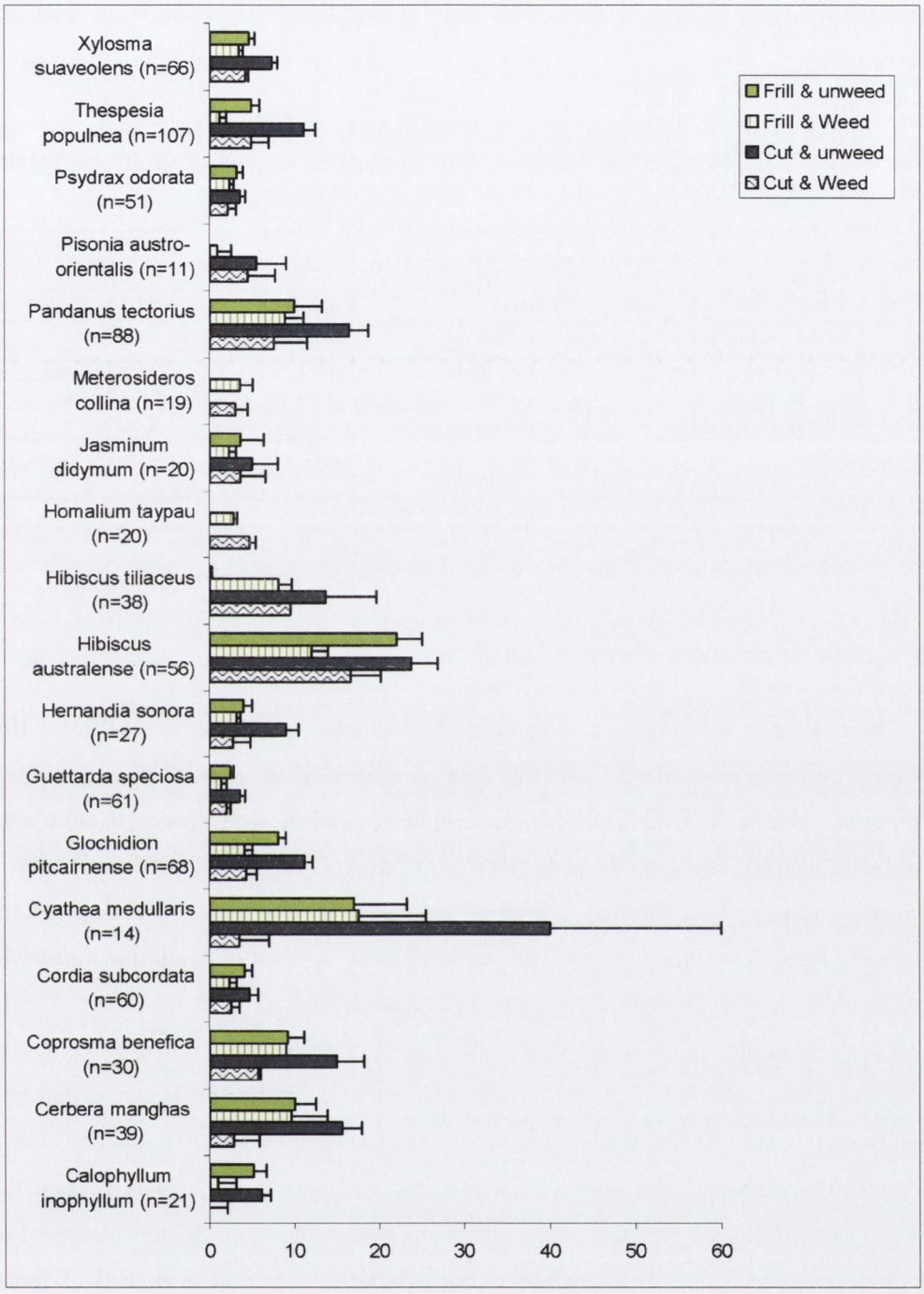


Figure 4.7 Mean basal diameter growth (+SE) across each of the experimental treatments applied during the ten month experimental period.

An analysis of variance was carried out using fourteen species excluding *Cyathea medullaris*, *Homalium Taypau*, *Meterosideros collina* and *Pisonia austro-orientalis* because they did not exist in all treatments. Data were log transformed to satisfy the criteria for similar heterogeneity of variances (Cochran's test=0.0982, ns). Basal diameter growth varied significantly with species ($p \leq 0.0001$), *Syzygium jambos* treatment ($p \leq 0.03$) and weeding ($p \leq 0.0001$). The interaction between species and treatment ($p \leq 0.0443$), treatment and weeding ($p \leq 0.0006$) and between species,

treatment and weeding ($p \leq 0.0017$) were also found to have significant effects on basal diameter growth (Table 4.9)

Table 4.9 ANOVA table for significance tests of treatments on planted species basal diameter growth. With individual species; *Syzygium jambos* treatment; and weeding treatment as significant factors influencing basal diameter growth

Source	*SS*	*DF*	*MS*	*F*	*P*
Species (Sp)	173.08	13	13.31	9.70	0.0000 **
Treatment (Tr)	6.19	1	6.19	4.51	0.0359 *
Weeding (We)	58.63	1	58.63	42.71	0.0000 **
Sp x Tr	32.94	13	2.53	1.85	0.0443 *
Sp x We	16.78	13	1.29	0.94	0.5142 ns
Tr x We	17.04	1	17.04	12.41	0.0006 **
Sp x Tr x We	49.84	13	3.83	2.79	0.0017 **
Residual	153.75	112	1.37		
Total	508.28	167			

** indicates significance at $p<0.01$; * indicates significance at $p<0.05$ & ns=not significant

4.3.5 Native tree seed experiment results

The success of direct seeding three native tree species was negligible, at around 10% (Table 4.10). *Hibiscus tiliaceus* germination success was low and germination of *Homalium taypau* and *Meterosideros collina* were both unsuccessful. *Hibiscus tiliaceus* was the only species to germinate of the three species sown in $2x2m^2$ grid boxes at seven of the eleven sites sown (Table 4.10).

Percentage germination of *Hibiscus tiliaceus* was low in the seven sites and ranged from nine to one percent (Table 4.10) which was lower than anticipated as seed grown in the nursery in trays in seed and potting compost in the propagation frame showed high germination rates (>75%).

Homalium taypau also germinated well (>95%) in the nursery when sown in trays with seed and potting compost and placed in the propagation frame so it can be assumed that site conditions were not favourable for its germination. *Meterosideros collina* also germinated (>60%) in the nursery in trays with seed and potting compost but seedlings failed to survive past the cotyledon stage. These results suggest that direct sowing of native tree species after treatment of the alien invasive tree species *Syzygium jambos* is not a successful or viable method of forest restoration on Pitcairn Island.

Table 4.10 Germination success of three native tree species directly sown into 2x2m^2 grids in sites where *Syzygium jambos* was treated.

Plot No. & Location	*Hibiscus tiliaceus* (n=100) No. Germinated	*Homalium taypau* (n=10) No. Germinated	*Meterosideros collina* (n=200) No. Germinated	Total Percentage Germination per site (n=310)
Plot 1 Flatlands Camp	5	0	0	1.61
Plot 2 Pulau	9	0	0	2.9
Plot 7 No Boar	7	0	0	2.25
Plot 8 Corner at the Hollow	9	0	0	2.9
Plot 9 Devil's Elbow	0	0	0	0
Plot10 Up the Flat	0	0	0	0
Plot13 Charles Aute	0	0	0	0
Plot14 Graveyard 1	1	0	0	0.32
Plot 15 Graveyard 2	0	0	0	0
Plot 16 Flatlands Mango	1	0	0	0.32
Plot21 Graveyard 3	2	0	0	0.64
Total % Germination	**34**	**0**	**0**	**10.9%**

4.3.6 Soil restoration success

Soils collected in 2003 before *Syzygium jambos* control treatments commenced were analysed as outlined in Chapter 2. Further soil samples were collected from plots during March 2005 and analysed for pH and soil organic matter using procedures outlined in Section 2.2.4 in December 2005 in the laboratories at Trinity College Dublin.

An analysis of variance was performed on the results of soil samples taken in 2003 and 2005 in *Syzygium jambos* plots before and after control and restoration experimental treatments and soil results from the 43 native forest community plots of Kingston & Waldren (2003). The percentage organic matter content of soils in *Syzygium jambos* plots after control and restoration efforts were similar to the organic matter percentage recorded before treatments and no significant differences were found between native forest community plots, *Syzygium jambos* plots before experimental treatment and after experimental treatment with an analysis of variance test ($F_{[2,126]}$ 3.86; p=0.02; n=129) (Table 4.11 & 4.12).

Table 4.11 Mean, standard deviation and standard error of percentage organic matter content in *Syzygium jambos* forest before experimental control and restoration efforts in 2003, Native forest communities defined by Kingston & Waldren (2003) and *Syzygium jambos* controlled and restored forest in 2006.

%OM	Native forest communities (Kingston & Waldren 2003)	*Syzygium jambos* forest before experimental treatments in 2003	*Syzygium jambos* forest at the end of experimental treatments in 2006
Mean	22.32	23.21	24.97
St. Dev.	5.24	3.20	4.54
St. Err.	0.12	0.07	0.68

The percentage organic matter was not found to differ significantly among native forest community quadrats and *Syzygium jambos* forest plots before the experimental treatment so this result was not unexpected (Table 4.12).

Table 4.12 ANOVA table for mean percentage organic matter content in *Syzygium jambos* forest before experimental control and restoration efforts in 2003, Native forest communities defined by Kingston & Waldren (2003) and *Syzygium jambos* controlled and restored forest in 2006. Data were normally distributed (Cochran's test =0.4,ns)

Source	*SS*	*DF*	*MS*	*F*	*P*
% OM	155.96	2	77.98	3.86	0.02 *
Residual	25.47.96	126	20.22		
Total	2703.95	128			

*p< 0.5

The difference in pH values recorded in treated plots in 2003 and 2006 was not found to be significantly different (Table 4.13 and Figure 4.13). At the end of the experimental period the difference in pH between the *Syzygium jambos* treated plots and the native forest community plots was still significant plots ($F_{[2,126]}$ 45.36; p=0.00; n=129) (Table 4.12 & Figure 4.8).

Table 4.13 ANOVA table for pH in *Syzygium jambos* forest before experimental control and restoration efforts in 2003, Native forest communities defined by Kingston & Waldren (2003) and *Syzygium jambos* controlled and restored forest in 2006. (Cochran's test =0.38,ns)

Source	*SS*	*DF*	*MS*	*F*	*P*
pH	21.66	2	10.83	45.36	0.000 **
Residual	30.09	126	0.23		
Total	51.76	128			

** p<0.001

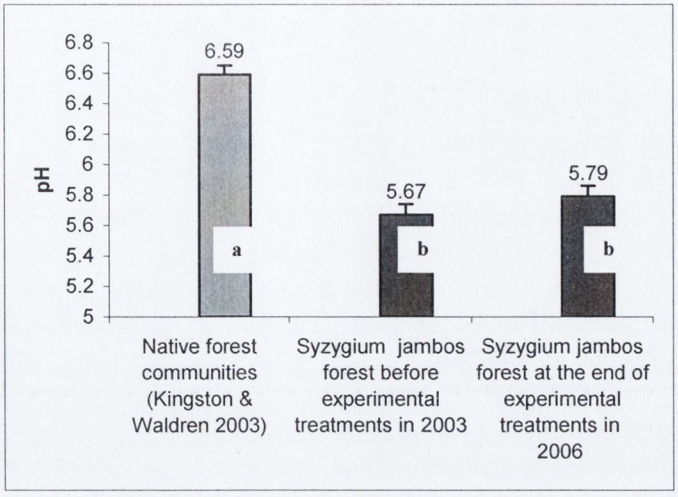


Figure 4.8 pH in *Syzygium jambos* forest before experimental control and restoration efforts in 2003, Native forest communities defined by Kingston & Waldren (2003) and *Syzygium jambos* controlled and restored forest in 2006. Bars with different letters are significantly different according to SNK post hoc test ($p<0.05$)

4.4 Discussion

4.4.1 Plant survival

Forest restoration is an increasingly important tool for species preservation and maintaining the diversity of forest communities (de Souza & Batista 2004). The results indicate the direct planting of trees and shrubs which were obtained from seed, cuttings and plant parts has the potential to aid the restoration of a species rich forest type after *Syzygium jambos* control efforts on Pitcairn Island.

After the experimental period, the survival rate obtained across the 28 different species chosen was 59.5% for the 1400 plants individually monitored. This figure is low in comparison to some other forest restoration a project, for example in Brazil, 89.9% survival was obtained for coastal forest restoration, two years after planting (Zamith & Scarano 2006). The plant survival results from the *Syzygium jambos* experimental plots were similar to results from forest restoration efforts in Hong Kong, where additional tree guards and weed mats were used to aid planted native tree species survival which was found to be 58% (Lai & Wong 2005). The results were also infinitely better than attempted native restoration of Seal Island in Western Australia where there was no long-term (>3 years) survivorship after plantings (Rippey *et al.* 2000).

Plant survival was highest in plots where *Syzygium jambos* was frilled treated *in-situ* and the plot subsequently weeded. The predominant cause for plant mortality in subplots was desiccation. This phenomenon was also noted in other restoration studies, for example in Mexican cloud forest, the tree seedlings planted had higher percentage survival rates inside forest fragments, the high temperatures, increased light and lower relative humidity in plantings outside forest fragments were considered the main cause of mortality (Alvarez-Aquino *et al.* 2004). In forest restoration in Uganda, four native tree species had 100% mortality in exposed large gaps in forest sites (Chapman *et al.* 1999). The results from Mexico and Uganda differ from forest restoration efforts in cooler climates like North America, where survival of planted native seedlings was higher in plots where the invasive shrub *Lonicera maackii* was cut and removed from the sites (51%), compared to those where *Lonicera maackii* was frilled and left in-situ (45%) (Hartmann & McCarthy 2004).

Goats decimated the planting at three experimental sites (Sites 4, 5, and 6) on Pitcairn and browsing accounted for the loss of 207 of the 1400 plants planted. Browsing by mammalian herbivores is common cause of mortality in restoration schemes with cattle browsing affecting restoration efforts in Mexico (Alvarez-Aquino *et al.* 2004) and deer browsing in Massachusetts (de la Cretaz & Kelty 2002). Goats grazing have caused severe effects on the vegetation on Sante Fe and Pinta Islands in the Galápagos group, and goat removal from these islands brought about improved vegetation regeneration (Hamann 1979). Goats may also be facilitating the spread of *Syzygium jambos* on Pitcairn Island as

they are adverse to grazing it (*pers. obs.*) and prefer to graze on native vegetation, this phenomenon has been noted in Killarney, Ireland where deer grazing was facilitating the spread of the invasive species *Rhododendron ponticum* (Cross 1981). High survival rates were found for the majority of plant species and some of the shrub species which were used and had high survival rates are extremely rare and globally threatened species, for example, *Hibiscus australense, Xylosma suaveolens* and the endemic *Coprosma benefica* whose global population consisted of a mere 12 individuals in 1998 (see Chapter 5). These results suggest that restoration and recovery efforts for these species are a feasible and worthwhile conservation option if goat grazing is curtailed on the island.

4.4.2 Plant growth

Highest plant growth was recorded from the *Syzygium jambos* cut and unweeded treatment the mean value was 70.8cm, this treatment however had the lowest plant survival percentage (42%). Plots which were left unweeded in the cut and frill treatments had higher plant growth rates (70.8cm and 43.8cm respectively) than those in the weeded cut and frill treatments (25.9cm and 17.9cm respectively). These results in part agree with results obtained from studies on Barro Colorado Island in Panama, where species exhibited higher growth and survival rates in the sun (Augspurger 1984; Brokaw 1985).

While it is difficult to compare the growth rates obtained on Pitcairn Island with other studies due to the number of native species used, in general growth rates were found to be high for Pitcairn. A similar study in Brazil (Zamith & Scarano 2006) recorded average growth of 11.3cm/yr for eight native tree species the slow growth rate was attributed to the nutrient poor soil at, the high light intensity and wind action as it was a coastal site. The height growth results from the *Syzygium jambos* experimental plots were higher than results obtained from Veracruz, Mexico where four tree species were used, these species exhibited greater height growth in the higher light environments outside the forest, which was similar to the result obtained from *Syzygium jambos* cut treatment plots, though in Mexico the height growth was low 0.2 to 1.2cm yr^{-1}.

Meterosideros polymorpha in Hawaii survived and grew in *Acacia koa* sheltered sites only (Scowcroft *et al.* 2000; on Pitcairn a similar species *Meterosideros collina* also survived and grew in the shade of treated *Syzygium jambos* which were frill treated. The growth rate for this species in Hawaii was 7.6cm over nine months on Pitcairn in the experimental plots *Meterosideros collina* growth was just marginally lower at 6.94cm over 10 months.

The highest growth measurements over ten months were recorded for *Hibiscus australense,* this. The rapid growth indicates that it may be a useful species to help reduce secondary weed invasion in treated *Syzygium jambos* plots and it is also a globally threatened species found only on Pitcairn, Raratonga and the Austral Islands. *Psydrax odorata* also had a low growth increment over the ten

months. This species is cut annually from the wild for display as a "Christmas tree" on Pitcairn Island, the low growth recorded here suggests this practice is unsustainable.

4.4.3 Direct sowing of native tree for forest restoration on Pitcairn Island

The results of the native tree seed experiment showed an extremely low rate of seed germination and establishment for the native tree species used. The native tree species used for direct sowing were *Homalium taypau*, *Meterosideros collina* of which none were found to have germinated or survived after ten months, and *Hibiscus tiliaceus* of which 10% were found to have germinated and survived after ten months.

Some of the successes with direct sowing of forest trees in the Amazon (Camargo *et al.* 2002) were put down to the positive relationship between seed size and survival after one year, with larger seeds having higher germination and survival rates. In this study, the seeds of *Meterosideros collina* and *Homalium taypau* are very small, and even dust-like in the case of *Meterosideros collina.* The small seed size was perhaps the reason why there was no germination success with direct sowing of these species into the plots.

A study in Canada (Matthes *et al.* 2003), also lamented low success rates with direct sowing of the native forest tree *Thuja occidentalis,* very few of which germinated. It was noted in this study that trees require a longer time to become established from seed and that seed may in fact be a poor choice for restoring forest habitats (Matthes *et al.* 2003). In other studies it was noted that any seedlings which may germinate can be more susceptible to environmental fluctuations and do not resist long periods of adverse conditions (Camargo *et al.* 2002). In the *Syzygium jambos* experimental plots the three tree species used; *Meterosideros collina*, *Homalium taypau* and *Hibiscus tiliaceus* germinated well and grew in nursery conditions all species had germination rates in excess of 60%, the nursery conditions provide regular watering, no predators and no competitors unlike the exposed plot conditions may not have favoured there survival.

Direct seeding is a generally favoured method for large scale forest restoration as seeds require low costs and effort to procure and plant (Harker *et al.* 1993) though the use of cutting material has been an underutilized method for restoration (Guerrant 1996). This view that direct seeding is more useful was also evident from studies from the islands of Western Australia where future plans for restoration efforts included plans to collect seed of native plants and hand-seeding them into tilled and untilled areas (Rippey *et al.* 2000). The results of this study refute the usefulness of direct seeding as a useful method for forest restoration. Though to be definitive whether or not restoration from direct seeding is a realistic possibility can really depend on the species in question (Camacho-Cruz *et al.* 2000).

4.4.4 Restoration Success

In broad terms, the planted species survival and growth in the *Syzygium jambos* experimentally treated plots can be considered a success with an increase in species diversity and structure in sites formerly dominated by *Syzygium jambos* Without restoration effort, secondary invasion by weedy species would occur in *Syzygium jambos* treated plots, which, as Cronk & Fuller (2001) point out, may negate the efforts of invasive species control as there is no point in eradicating an invasive species to have it replaced by a worse one. The lack of native seed in the seed bank and the small numbers of native species found regenerating in the treated plots also reinforce the need for pro-active restoration effort after *Syzygium jambos* control.

It will take some time before the larger scale processes such as soil ecology, plant dispersal and a self-containing community required to satisfy the criteria of a successful can be assessed (Ruiz-Jaen & Aide 2005). While attempts were made to grow a range of native and naturalized species, no attempt was made to make a replicate of the actual existing native forest communities; the *Meterosideros collina* forest community, *Pandanus tectorius* forest community and *Homalium taypau* forest community. These species however, were planted in sites and many survived. Efforts were also made also to ensure the planted material would form a forest structure with native tree and shrub species planted at each site. The planned structure will hopefully start to resemble the native forest structure with a tree layer, shrub layer and ground flora layer.

The ground flora in this case was not added as it was felt that ground flora species would arrive once favourable conditions and forest structure were restored. The appearance of native fern species such as *Nephrolepis cordata* and *Doodia media* after 10 months in some of the experimental sites indicates that this may be the case into the future. Plants are widely used as a surrogate for restoration success as measurement of vegetation structure can be easily monitored into the future (Ruiz-Jaen & Aide 2005). A surrogate measure for biological processes, such as the measure of organic matter content in soils, is commonly used in restoration studies (Ruiz-Jaen & Aide 2005). In this experiment there was no change in the soil organic matter content after replacement of *Syzygium jambos* with native and naturalized species, although, the experimental period of 20 months is a very short time period to access any change in terms of soil processes. pH at the end of the experimental treatment also was similar to the initial recorded pH values obtained in *Syzygium jambos* forest. As the native and naturalized species continue to grow and deposit leaf litter, it would be expected that pH values would change and become closer to values found in the native forest communities on Pitcairn Island,

4.5 General conclusions

Forest restoration is considered an important aspect of maintaining both threatened species and plant diversity. The forest restoration efforts in this project focused on building a forest structure and improving species diversity which would prove a refuge for both timber and fruits to the islanders and help restore many of the islands threatened plant species. The island species grown during this project proved amenable to general plant propagation techniques. Plant survival was determined mostly by plot conditions and the presence of goats. The plot conditions can be ameliorated somewhat by using the frill technique outlined here, the presence of goats on the island however, can not be so easily dealt with. Goats have caused much devastation of island floras, for example, on the Galápagos Islands. The problem of goats on Pitcairn Island will thwart any further forest restoration efforts by grazing unless they are exterminated; also there is little hope of retaining much of the remaining patches of natural vegetation on the island the source of all propagation material for forest restoration efforts on the island.

"Nature only uses the longest threads to weave her patterns, so each small piece of her fabric reveals the organisation of the entire tapestry"

Feynman 1980

Chapter 5

Species Recovery and Monitoring

5.1 Introduction

5.1.1. Species based Conservation

Maintaining species and preventing extinction is at the core of conservation biology. Every species has an intrinsic value in conservation biology but the species which merit more attention from conservation biologists are those most threatened with extinction.

The features of island biota which make them more susceptible to extinction range from existing species poverty, reduced competitive ability, the existence of small populations with low genetic variability with the lack of adaptability to change (Loope 1988; Cronk & Fuller 1995; Cronk 1998) and island endemic species in particular have higher extinction rates than non-endemic species (Frankham 1998).

Species vary in their taxonomic uniqueness and species that have no closely related species are generally considered more important than species with many close relatives (Hunter 1996). It has been noted that rare and uncommon species can make significant ecosystem contributions as in the majority of communities most species exist in relatively low abundance (Rabinowitz *et al.* 1986; Howe 1999).

The IUCN (The International Union for the Conservation of Nature and Natural Resources) regularly publish Red list categories and criteria that provide the framework for classifying and discussing the species of concern to conservationists. The categories were initially based on Mace & Lande's (1991) system for quantifying the risk of extinction for different taxa. In the current version of categories and criteria (IUCN 2.1 2001) there are 9 categories, extinct (**Ex**), extinct in the wild (**Ew**), critically endangered (**Cr**), endangered (**En**), vulnerable (**Vu**), near threatened (**Nt**), Least concern (**Lc**), data deficient (**Dd**) and not evaluated (**Ne**). Specific criteria to each category apply, enabling a taxon under assessment to be assigned to one of the categories.

There are many critics of the IUCN scheme, some point out that the area of occupancy of a species holds too much weight which results in species which have a small area of occupancy (IUCN critically endangered threshold is $<10km^2$) being listed as critically endangered (CR) (Kingston 2001). The IUCN criteria also do not account for species with skewed demographic structure, unusual life-histories, or the actual stability of habitats (Keith 1998; Waldren *et al.* 1995b). Having both a small area of occupancy and rarity can be a natural state, for example *Eumorphia swaziensis* Compton, a member of the Asteraceae occurs in two populations with less than 1000 individuals, whose sole

habitat is one mountain in Swaziland. This taxon has most likely always been rare but perhaps is not threatened (Lucas & Synge 1978).

Rabinowitz (1981) expanded the definition of rarity on the basis of three main characteristics: *1. Geographic range* - rare because they are found only in a small area e.g. small island, mountain, lake, etc. *2. Habitat specificity* -species which only occur in uncommon types of habitat, for example, desert springs, caves etc. and *3. Small local population size.* Although species with small population numbers may naturally be rare, and may not immediately be threatened, but they need to be monitored carefully because there status can shift quickly from secure to endangered (Hunter 1996).

Kingston & Waldren (2005) assigned both IUCN Red Data threat categories and local threat numbers based on modifications of the approaches of Rabinowitz *et al.* (1986), Curtis and McGough (1987) and Waldren *et al.* (1999) to all the indigenous and endemic species on Pitcairn Island. They found that 60% of the indigenous flora is threatened on the island and over 20% is globally threatened.

5.1.2 Species monitoring and recovery

Considering the sessile habit of plants and the ease in which they can be counted, data on population sizes are surprisingly sparse in the literature (Barrett & Kohn 1991). Knowledge on the number, size and structure of populations is fundamental in assessing risk extinction (Keith 1998), planning their recovery (Tear *et al.* 1995) and critical in characterising the biotic interactions and habitat requirements of a species (Burgman *et al.* 1988, Simberloff 1988, Brussard 1991).

Monitoring can provide baseline measurements about the spatial occurrence and population status of rare plant species. Recovery efforts for rare species require a "top down" approach; a three tier monitoring and recovery effort was suggested by Menges & Gordon (1996):

1. Locate, map distribution and assign threat categories.
2. Assemble the necessary demographic information to discover the current conditions of a species and find out if the population is increasing, decreasing or stable.
3. Monitoring of marked individuals for a quantitative assessment of survivorship growth and fecundity. Investigate the causes of variation in the demographically sensitive life history stages and the biological causes of variation in those life history changes that have a major impact on demographics (Figure 5.1).

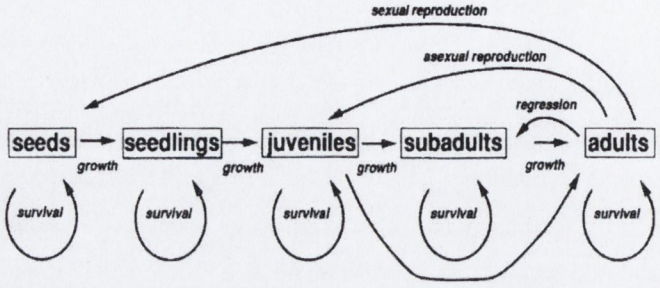


Figure 5.1 Hypothetical plant population with five life history stages and the influence of survival and reproduction on the population demographics (Schemske *et al.* 1994)

When populations are extremely small they can be estimated by direct count of all individuals without appreciable error. When populations are >50, random stratified density sampling offers a repeatable, simple and efficient method (Keith 2000). Though it is thought that estimates of population size based on contemporary population size are often a poor surrogate for long term effective population size, most population studies are based on only one year and few encompass more than 3 years (Reed 2005).

The population structure and not size is of relevance for conservation purposes as it is not the same as the number of individuals in a population. Population structure reveals the proportion of individuals in a population capable of reproducing. This is recognised by the IUCN (1994) which excludes immature, reproductively suppressed and senescent individuals from estimates of population size used in Red List assessments. The size of the breeding population is rarely equal to the total population size.

A useful population parameter first introduced by Wright (1931) is N_e the "effective population size". The effective size can be estimated from the actual number of breeding individuals, when factors such as mating system, sex ratio and variation in fertility are known for populations of plant species, which in essence gives the effective breeding population. N_e may be considerably smaller than the census number (Jain & Rai 1974; MacKay 1981). The demographic patterns of a population may change over time which might be caused by succession (Horvitz & Schemske 1986) coupled with demographic and environmental stochasticity (Menges 1992).

A rigorous approach to population surveying with priority and relevance to the problems in hand is essential (Murphy 1991). Critics conclude that many field surveys neglect to estimate population size or record critical life history observations of the target species. The fact that very little guidance exists on how to design and implement field surveys of plant populations in ways that provide meaningful answers was highlighted by Keith (2000).

In addition to understanding the populations and biology of endangered plants, conservation biologists must be fully informed of the political and economic factors that can limit the feasibility and effectiveness of recovery efforts. Substantial progress can be made if moderate funds are made

available to develop realistic and efficient guidelines for the management of rare species. Critics of research programmes have highlighted the fact that in many instances researchers fail to provide clear guidelines regarding the most valuable and cost effective approaches for managers involved with endangered species recovery (Schemske *et al.* 1994).

5.1.3. Species conservation, genetic diversity and population size

The genotype is of most interest and significance to geneticists and conservation biologists. The significance of size of populations to their breeding structure, genetics and evolutionary dynamics was first recognised by Wright (1931, 1938 & 1946). Proponents of a population genetic approach stress that understanding the organization of genetic diversity is key to the long term survival of species since genetic variation is a requisite for evolutionary adaptation (Berry 1971; Lande & Barrowclough 1987; Vrijenhoek 1987; Hamrick *et al.* 1991). Of the various problems which face a declining population, the loss of genetic variability and inbreeding depression are the most detrimental (O'Brien 1994; Amos & Balmford 2001).

Inbreeding depression seems the factor most likely to exacerbate population decline and hasten extinction particularly where the reduction in population size has been very great and under conditions which are stressful rather than benign. However, some recent evidence suggests that strong purging during a species "bottleneck" can increase fitness by decreasing the genetic load (Saccheri *et al.* 1996). This can be particularly true of island colonists, if they survive colonisation and develop substantial populations then by implication they are capable of surviving a "bottleneck".

The effects of inbreeding on fitness in plants can also be more variable than that in animals due to diverse reproductive systems and population structures (Charlesworth & Charlesworth 1987). Generalizations on the harmful effects of inbreeding and recommendations on the numbers required to maintain viable populations need to be modified for plants because many species of plants regularly inbreed at least to some degree. In some cases it may be more important to determine whether outbreeding depression, the fitness decline that can result from hybridization (Templeton 1996) occurs.

Maintenance of genetic variation is considered essential for the long-term survival of a species (Frankel & Soule 1981). Most models of the rate of loss of genetic variation assume that all genotypes have equal fitness. The amount of heterozygosity remaining after t generations is $(1 - \frac{1}{2} N_e)^t$ with neutral models, where N_e is the effective population size. Based on these models it has been estimated that effective population sizes of at least 50 are needed to avoid harmful loss of genetic variation in the short term (Franklin 1980; Soule 1980; Frankel & Soule 1981) though this number has been debated in the literature review (Simberloff 1988). A recent consensus on the subject states that populations should be managed to maintain 95% of their original fitness and management programs should aim to

maintain the original genetic diversity (Reed 2005).The relationship between population size and extinction probability is not as clear cut for plant species. External factors such as environmental variation and dispersal rates which can be independent of size can be more important determinants of plant population extinction (Figure 5.2) (Schemske *et al.* 1994; Quinn & Hastings 1987; Robinson & Quinn 1988; Bowles & Apfelbaum 1989; Menges 1990).

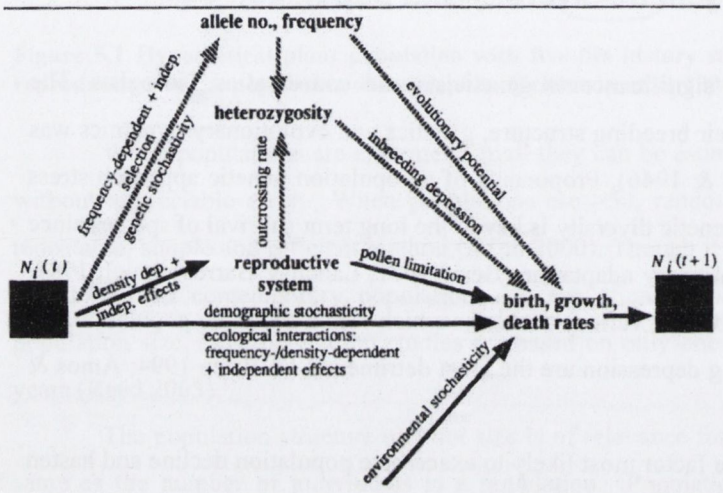


Figure 5.2 The ecological (solid arrows) and genetic (hatched arrows) processes that affect plant population size (Schemske *et al.* 1994).

5.1.4 Species conservation and use of AFLP molecular markers

The use of molecular markers in species conservation has become increasingly popular (see Chapter 1) (Karp *et al.* 1996; Ouborg *et al.* 1999). The AFLP technique has proven to be particularly useful as no information on the target genome sequence is required before analysis, and primer combinations usually reveal numerous and reliable genetic markers (Meudt & Clarke 2007; Bensch & Åkesson 2005; Tero *et al.* 2003; Hodkinson *et al.* 2000). AFLP randomly samples the entire genome and can provide an overall picture of the diversity of the samples being examined. It is based on the detection of DNA restriction fragments by PCR amplification (Vos *et al* .1995; Zabeau & Vos 1993) and can reveal high degrees of polymorphism and is highly reproducible.

AFLP involves restriction digestion of the genomic DNA, and is followed by a selective PCR amplification of the restricted fragments. The amplified products are separated on a sequencing gel in bands and can be visualised. The technique has been adapted for automation using fluorescently or radioactively labelled primers (Karp *et al.* 1996; Meudt & Clarke 2007). Fluorescent bands are scored as present or absent and these can be converted into measurements of similarity or dissimilarity.

Sørenson distance, for example, can provide a method of discovering how genetically distant individuals or taxa are from each other. The unique genetic fingerprint of fluorescent bands in the form of multi-locus profile can distinguish between even closely related genotypes. The band difference between genotypes can be used to provide information on how much diversity is present, and whether it is distributed among or between populations, relatedness can also be estimated from band sharing.

AFLP has proven to have a wide range of applications ranging from cultivar and variety identification (Hodkinson *et al.* 2000; Ridout and Donnii 1999; Becker *et al.* 1995; Loh *et al.* 1999; Chao 2005) to diversity studies and phylogenetic reconstruction (Hodkinson *et al.* 2000; Hodkinson *et al.* 2002), and has proven particularly useful in studies on rare and endangered plants (Gaudeul *et al.* 2000; Coart *et al.* 2003; Zawko *et al.* 2001; Nielsen 2004; Forrest *et al.* 2004; Peakall *et al.* 2003; Palacios *et al.* 1999; Krauss 1999; Martin *et al.* 1999; Krauss *et al.* 2002; Keiper & McConchie 2000; Juan *et al.* 2004; Tero *et al.* 2003).

5.1.5 Species conservation and use of the trnL-f gene region for resolving taxonomy

The differing genes and gene regions of the chloroplast genome provide information of different quality and quantity for systematic and phylogenetic investigations. A brief overview of the three of the most commonly and widely used regions is given here, and further information on the differing regions and their utilities can be obtained from Soltis, Soltis & Doyle (1998).

The most widely used gene, *rbc*L, is located in the large single copy region of the chloroplast genome and encodes the large subunit of ribulose 1,5-biphosphate carboxylase/oxygenase (RUBISCO). It has been widely used for investigations at family level and above (Soltis, Soltis & Doyle 1988). Similarly the gene *atp*B, which encodes the β subunit of ATP synthase, an enzyme that couples proton transfer across membranes with the synthesis of ATP (Hoot *et al.* 1995), has been used to augment *rbc*L-based topologies and provides better resolution of relationships (Chase, Soltis, Olmstead *et al.* 1993).

The *mat*K (formerly ORFK) gene is one of the more rapidly evolving regions, encoding a maturase involved in splicing type II introns from RNA transcripts (Wolfe *et al.* 1992). This gene is appropriate for resolving intergeneric or interspecific relationships as it has a faster rate of evolution compared to *rbc*L and *atp*B. However, because of its rapid evolutionary rate additional sequencing primers have to be developed when investigating a group of interest (Soltis, Soltis & Doyle 1988).

In this study the intergeneric spacer between two tRNA genes *trn*L intron and *trn*L-F intergenic spacer (*trn*L-F) was sequenced (Figure 5.3). This sequence is non-coding and evolves at a rate that is similar to other frequently used cpDNA regions, though *mat*K evolves faster (Gielly &

Taberlet 1994; Fujii *et al.* 1997; Kajita *et al.* 1998), though the *trn*L intron and *trn*L-F intergenic spacer (*trn*L-F) is suitable for elucidating intraspecific and intrageneric relationships.

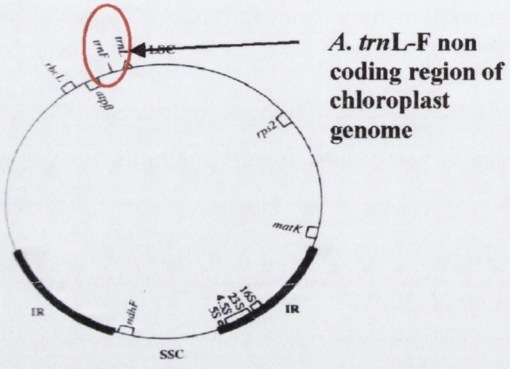


Figure 5.3 A. Diagram of Chloroplast genome illustrating the chloroplast regions used in taxonomic and systematic studies. The 800 base pair *trnL-F* intergenic spacer region is highlighted in red

There are two steps in obtaining *trn*L-F sequences. Step one involves amplification of the target DNA through use of the Polymerase Chain Reaction (PCR). The primer pairs used (C- primer forward and F-primer reverse) give specificity to the PCR reaction. Step two involves purification of the PCR product by using a cycle sequence reaction which includes terminators which stop DNA synthesis and have a fluorescent dye attached. These terminators are added at random so the sequence can be primer +1 base pairs or primer +768 base pairs. The fluorescent dye allows identification of sequence variation between individuals. The fluorescently labelled sequences can be then characterized in an automated sequencer.

The advantage of using *trn*L-F region is that universal primers were designed which work across many groups for this non-coding region by Taberlet *et al.* (1991). Chloroplast *trn*L-F sequences have been used to resolve relationships in *Elymus* (Liu *et al.* 2005), *Plantago* (Ronsted *et al.* 2002) *Taraxacum* (Wittzell 1999), investigate the evolution and taxonomy of *Actinidia* (Ciprianai *et al.* 1998), investigating the extent of haplotype diversity of *Fraxinus excelsior* (Harbourne 2004) and study hybridization in *Miscanthus* (Hodkinson *et al.* 2002).

5.1.6 Aims

The overall aim of this chapter is the conservation of threatened Pitcairn plants and to recommend actions for further recovery measures and future management. The specific aims are:

1. Investigate the genetic diversity within populations of the endemic species, *Coprosma benefica* and *Abutilon pitcairnense.*
2. Investigate the taxonomic status of *Myrsine* aff. *niauensis* on Pitcairn island.
3. Investigate the population demography of *Lastreopsis* c.f. *pacifica* and *Haloragis* sp.
4. To locate, map and assess the conservation status of all individuals and populations of the above listed species.

5.2 Material and Methods

5.2.1. Population monitoring and census measurements

A field survey was carried out for each of the following five species: *Coprosma benefica*, *Abutilon pitcairnense, Myrsine* aff. *niauensis, Lastreopsis* c.f. *pacifica* and *Haloragis sp.*. The location of the population or individual was recorded using GPS (Garmin *etrex*) along with general habitat and environmental measurements such as location (location name on Pitcairn map), aspect (compass), altitude (altimeter), slope (clinometer; Silva *Clinomaster*) and vegetation type (Table 2.7).

A description, size and stage of development (juvenile, mature, fertile) was recorded for each individual. Size classes appropriate for each taxa were derived from field observation of each species. Changes in population size can be expressed as the growth rate, λ, which was defined by equation 5.1.

$$\lambda = N_{t+1} / N_t$$

where N_t *is the population size at year* t *and* N_{t+1} *is the population size the following years.*

Equation 5.1 Population Growth Rate λ

A conservation management assessment was carried out for each population based on quality of the population, the condition of the habitat, the long term prospects for the species in question and the nature of site protection. An overall score was obtained from the assessment which pertains both to the population and site in which the species was found. The system was updated from earlier suggested survey and monitoring assessments by Given (1994).

- **Quality** - consider the size and productivity of the population and the vigour of individuals. (**1**= >100 individuals; **2**=51-99 individuals; **3**=<50 individuals; **4**=<10 individuals)
- **Condition** - Habitat pristine or degraded (**1**=excellent; **2**=good; **3**=marginal; **4**=poor).
- **Long term prospects** -The long term prospects for continued existence of this population at the indicated site. (**1**=excellent; **2**=good; **3**=marginal; **4**=poor.)
- **Site protection** - Population protected from extrinsic human factors (**1**=excellent; **2**=good; **3**=marginal; **4**=poor).
- **Score** - a summary of all above listed factors with summary value (**4**=excellent; **8**=good; **12**=marginal;**16**=poor)

5.2.2 Sample collection for genetic investigations

Generally investigations into the genetic diversity of populations rely on sampling a large number of populations across the range of the species (Kay & John, 1997). In this case the entire populations of the endemic species investigated reside on Pitcairn Island. Samples were collected from every known individual in the populations of *Coprosma benefica*, *Abutilon pitcairnense* and *Myrsine* aff. *niauensis*; this was easily achieved as population numbers are extremely small.

Leaves were sampled from each of the individual plants from actively growing fresh shoots and from near the shoot tips in random locations on the plants. When collecting the leaf material, leaves showing signs of disease, virus and/or fungal infection were avoided. The leaf samples chosen were torn into 10mm sections to speed the drying and desiccation process and reduce the amount of silica gel required (Chase & Hills 1991), and were stored in Type III indicating silica gel for genetic analysis (Sigma-Aldrich) in polythene zip lock bags immediately after collecting.

Silica gel was used as it prevents the degradation of the DNA through the removal of moisture from tissue (Chase & Hills 1991) as it was not possible to use fresh material. Silica gel was periodically replenished when the indicating colour pink denoted the silica gel was no longer able to absorb any more moisture from the sample leaf tissue. Vouchers specimens were prepared as herbarium mounts and deposited in the herbarium of the University of Dublin, Trinity College.

5.2.3. Extraction of total genomic DNA

The protocol used for the extraction of total genomic DNA from silica dried leaf material was a modified version of *Qiagen DNeasy Minikit* extraction protocol. This method was chosen for its reliability in the production of sufficient quantity and quality of DNA for PCR reactions based on previous molecular studies in the Botany Department, Trinity College molecular laboratory (Kingston 2001; Smith 2004) using a variety of taxa. Using Minikits to extract DNA offers advantages over manual methods, such as, organic extraction. The Minikit also provides for the removal of contaminants such as polysaccharides and phenolics which can inhibit the action of restriction endonucleases and reduce the efficiency of the PCR reaction (*Qiagen Minikit Extraction* Booklet).

Protocol for the Extraction of Total Genomic DNA
(QIAGEN DNeasy Plant Mini kit and Mixer Mill MM300)

A 1.5 ml tube was filled with 50 µl of elution buffer (Buffer AE) and placed on a heating block at 65^{0}C. Another clean, labelled 1.5ml tube was filled with 12mg of dried leaf sample, taking care to use tissue from areas between the main leaf veins, and a metal ball was placed in the tube with the sample and 400 µl of lysis buffer (Buffer AP1) and 4 µl of RNAase A stock solution were added.

The labelled tubes were then placed on a mixer mill rack (Qiagen MM300) for 1.5 minutes at 30 H_Z. The racks were turned and milling repeated three times in order to sufficiently break down the tissues in the leaf. Care was taken to ensure each rack was balanced before starting the machine.

The labelled samples were then placed in a micro-centrifuge and pulsed to 3000rpm[11] which spins down froth formed in mixer mill from the lids of the tubes. The tubes were tapped and mixed gently, and incubated for 15 minutes on a heating block at 65°C (the protocol suggests 10 minutes but the additional time was found to aid cell lysis especially in tough leaves, leaves from herbarium samples and leaf material from which it was difficult to obtain DNA using standard methods).

Precipitation buffer (Buffer AP2) was added to each labelled tube and the samples were placed on ice for 10 minutes (original protocol suggests 5 minutes, the additional time allows for the formation of more precipitates with difficult material). The samples were then centrifuged for 5 minutes at 13,000 rpm to spin down precipitates formed. The supernatant from the labelled tubes was then placed in labelled QIA shredder spin columns and centrifuged at 13,000 rpm for 2 minutes.

The lysate which was in the bottom of the tube was transferred to a clean 1.5 ml tube and the volume of lysate was recorded. Binding buffer (Buffer AP3/E) was added at 1.5 times the recorded volume of lysate for each sample and the buffer was mixed with the sample by drawing in and out with a pipette three times. DNeasy spin columns with collection tubes were labelled and 650 µl of the lysate and buffer mix were added to the spin columns and centrifuged at 9,000 rpm for 1 minute. The flow through was discarded and any remaining sample and buffer mix was added and centrifuged at 9,000 for 1 minute.

Purification of Total genomic DNA using QIAGEN DNeasy Mini plant Kit

The spin columns were placed in new labelled 2ml tubes and 500 µl of wash buffer (Buffer AW) was added and centrifuged at 9,000 rpm for 1 minute. The flow through was discarded and 400µl of wash buffer (Buffer AW) was added and centrifuged for 2 minutes at 13,000 rpm. The spin column was placed in a new 1.5 ml labelled tube and 50 µl of preheated elution buffer (Buffer AE) was added to each sample and left at room temperature for 15 minutes (original protocol suggests 5 minutes), repeated experiments found that the longer the sample was left the greater the quantity and quality of DNA obtained. The samples were centrifuged at 8,000 rpm for 1 minute and the spin columns were discarded. The stock DNA obtained was stored at -20°C.

[11] **rpm:** revolutions per minute

5.2.4 Quality and quantity assessment of total genomic DNA

Two different methods were used to assess the quality and quantity of total genomic DNA, ethidium bromide quantification and spectrophotometric determination of DNA.

Protocol for assessing quantity and quality of total genomic DNA using ethidium bromide quantitation

Ethidium bromide fluorescent quantification is a crude assessment and was determined using electrophoresis on an agarose gel containing ethidium bromide (it works on the principle that stained DNA fluoresces in the presence of UV light). The assumption with this method is that a direct correlation can be made between the quality of DNA present and the degree of fluorescence using a series of standards. The advantage of this method is that as little as 1-5ng of DNA can be detected.

Approximately 80ml of gel solution is needed for a medium size gel (Horizon 10 x 14 System). The gel was made by adding 1.2 g of agarose to 100 ml of 1 x TBE[12], these were placed in a Duran Bottle with the lid left loose and heated in a microwave on full power for 2 minutes. Before proceeding, it was ensured that all agarose had melted. 1.5 µl of ethidium bromide was added to the Duran bottle and swirled to mix well. The mix was allowed to cool until it could be held by hand.

The casting boat was prepared for the gel by placing the boat on a level surface (checked with spirit level) taping the edges of the boat with masking tape and adding combs which allow small wells to be set into the gel. The gel mix was poured into the boat once the mix had sufficiently cooled and the gel was allowed to set for at least 30 minutes. After 40 minutes the masking tape and combs were removed and the gel was placed in an electrophoresis tank and covered with 1 x TBE.

6.5 µl of total genomic DNA of each sample was mixed with 2 µl loading dye[13] and loaded into the gel wells. The electrophoresis tank was connected to the power supply (EC-Apparatus Corporation EC 105 power pack) by leads and ran for 20-30 minutes at 125 V. The negatively charged DNA migrated to the positive anode. Then the gel was removed from the electrophoresis tank and placed in a UV light box (Dual Intensity Transilluminator UVP) and a digital image of the gel was taken using a Kodak Digital Science DC120 Zoom Digital Camera and the images imported into Scientific Imaging System from Digital Science (Kodak ID 2.0.2) gel photography software (Figure 5.4).

---

[12]**1 x TBE** : Tris (10mM), Boric Acid and EDTA (ethylene-diamine-tetra acetic acid disodium salt at a pH 8 (pH is controlled by HCL addition)

[13]**Loading Dye**: 0.25 % Bromophenol Blue :40% Sucrose

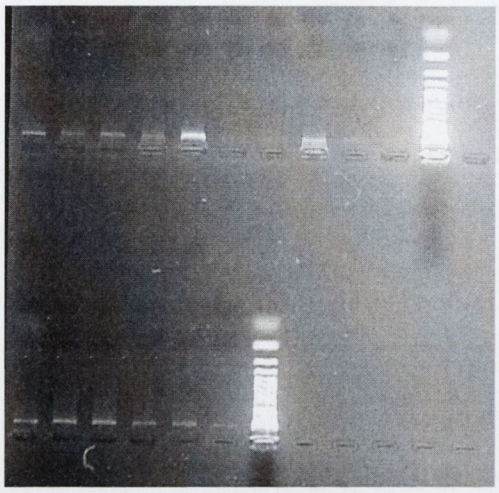


Figure 5.4 Ethidium bromide fluorescence indicating the presence of Total genomic DNA . The brighter bands show total DNA of higher concentration. The long fluorescent bands at the end are DNA ladders which can used to measure the length of the DNA fragment.

Protocol for assessing quantity and quality of Total genomic DNA using spectrophotometric determination.

Spectrophotometric determination of DNA measures the UV absorbance of nucleic acids. The spectrophotometer was calibrated such that an absorbance of 260 nm (A_{260nm}) is equivalent to 50ngμl^{-1} of double stranded DNA. The ratio between the absorbance at 260nm and 280 nm provides a quantitative estimate for the purity of DNA. Pure preparations of DNA have absorbance of 260nm/280nm of 1.8 and 2.0 respectively (Eppendorf Biophotometer Manual).

A spectrophotometer (Eppendorf Biophotometer) was used and plastic disposable cuvettes (Eppendorf UVette®) were loaded with a blank (5 µl TE $_{0.1}$ Buffer[14]: 45 µl sterile water) and total genomic DNA sample (5 µl Total DNA sample: 45 µl of sterile water). Each sample was thoroughly mixed and care was taken to ensure no bubbles remained as these can interfere with the light path and subsequent readings. The cuvettes were inserted into the spectrophotometer which was set at a wavelength of 260nm and a light path of 10mm was selected. The sequence used to load samples onto the machine was blank followed by sample. The absorbance of 260nm light through each sample was read directly from the machine display which gave DNA concentration in ng/µl (equivalent to µg/ml).

5.2.5 Protocol for AFLP genetic marker analysis

As all the samples used had low concentrations of DNA (~ 250ng/µl) they were left undiluted for all reactions. There are three main reactions for AFLP analysis: Restriction of the DNA and Ligation of Adapters, Pre-Selective Amplification by PCR and finally Selective Amplification by PCR. All AFLP

[14] **TE $_{0.1}$ Buffer**: 2ml 1 M Tris-HCL (pH8), 40 µl 0.25 M EDTA, 97.96 ml H_20

reactions were carried out using Applied Biosystems AFLP 1 Kits. When preparing the master mixes an extra two volumes of each reagent were added to allow for pipette error. Negative (sterile water in place of DNA sample) and positive (DNA with a clear consistent fingerprint) Asparagus DP3, (Smith 2005) controls were used in all experiments.

Restriction-Ligation Reaction

PCR tubes were labelled one per sample and 2.75 µl of total genomic DNA from each was aliquoted and placed on ice while the Master Mixes were made up. The first master mix (Master Mix1) was made from components listed in Table 5.1.

Table 5.1 Components of Master Mix 1 for Restriction-Ligation Step of AFLP analysis

Component	Volume per sample
T4 DNA Ligase Buffer[15]	0.05 µl
NaCl (0.5M)	0.05µl
BSA (1mg/ml)[16]	0.025µl
Mse I (10U/µl)	0.05µl
*Eco*RI (100U/µl)	0.025µl
T4 DNA Ligase with ATP[17]	0.005µl
Ultra pure H_20	0.295µl

Master Mix 1 was mixed thoroughly using vortex spin (Whirlimixer ™) and centrifuged at 3,000rpm for 10 seconds. Master Mix 1 was then placed on ice while the second master mix and adaptor pairs were prepared. *Mse*I Adaptor pair and *Eco*RI Adaptor pair were aliquoted at 0.5 µl per sample and incubated for 5 minutes at 95°C, left at room temperature for 10 minutes and centrifuged 3,000 rpm for 10 seconds before addition to the second master mix.

The second master mix (Master Mix 2) was prepared using Master Mix 1, the *Mse*I adaptor pair and the *Eco*RI adaptor pair and extra components outlined in Table 5.2.

Table 5.2 Components of Master Mix 2 for the Restriction-Ligation step of AFLP analysis.

Component	Volume per sample
T4 DNA Ligase Buffer with ATP	0.5µl
0.5 M NaCl (0.5M)	0.5µl
BSA (1mg/ml)	0.25µl
Cooled MSEI Adaptor pair	0.5µl
Cooled EcoRI Adaptor pair	0.5µl
Master Mix 1	0.5µl

[15] **T4 DNA Ligase Buffer:** 300 mM Tris-HCL (pH.7.8), 100 mM $MgCl_2$, 100mM DTT & 10 mM ATP
[16] **BSA:** Bovine Serum Albumen
[17] **ATP:** Adenosine triphosphate

2.75µl of the combined mix (Master Mix 2) was added to each 2.75µl DNA sample and was mixed thoroughly by vortex and centrifuged for 10 seconds at 3,000rpm. The samples were then incubated for 2 hours at 37°C (Geneamp® PCR system 9700 Applied Biosystems).

The products of this restriction-ligation reaction were diluted by adding 94.5µl $TE_{0.1}$ buffer to each sample and thoroughly mixed. The diluted products were used immediately in the next pre-selective amplification step or stored at -20°C until required for pre-selective amplification.

Pre-Selective Amplification Reaction

A new set of PCR tubes were labelled and 1 µl of diluted restriction-ligation products were added to each and stored on ice while the master mix was being prepared (Table 5.3).

Table 5.3 Components of Master Mix for pre - selective amplification reaction for AFLP analysis

Component	Volume per sample
AFLP pre-selective primer pairs (Pre-amplification primers)	0.25µl
AFLP Core Mix [18]	3.75 µl

4 µl of pre-selective amplification master mix was added to 1 µl diluted restriction–ligation products and mixed thoroughly by vortex and centrifuged to 3,000 rpm for 10 seconds. The pre-selective amplification was then carried out using Geneamp® PCR System 9700 (Applied Biosystems) with heated lid following the procedure outlined in Table 5.4

Table 5.4 Reaction conditions for pre-selective amplification of restricted –ligated DNA fragments

Hold	Cycle			Hold	Hold
	Each of 20 cycles				
Extension	**Denaturing**	**Annealing**	**Extension**	**Final Extension**	**Soak**
72°C	94°C	56°C	72°C	60°C	4°C
2 minutes	20 seconds	30 seconds	2 minutes	30 minutes	∞

A check for successful amplification of samples was carried out using agarose gel electrophoresis using power setting of 4 V for 3-4 hours. A smear of product was visible after successful amplification. The pre-selection amplification products were then diluted with 95 µl $TE_{0.1}$

[18] **AFLP Core Mix:** Applied Biosystems AFLP 1 amplification core mix containing buffer, nucleotides and Amplitaq ® DNA polymerase

buffer. The products can then be used in the selective amplification reaction or stored until required at 4^0C.

Selective Amplification

Sets of PCR tubes were labelled, one for each primer pair combination. 1.5 µl of the diluted pre-selective amplification products were added to each tube. A master mix was prepared for each primer pair combination using the components outlined in Table 5.5.

Table 5.5 Components of master mix for selective amplification reaction for AFLP analysis

Component	Volume per sample
Primer *Mse*I (5mM)	0.5 µl
Primer *Eco*RI (1µM) & Fluorescent Dye	0.5µl
AFLP Core mix	7.5µl

8.5 µl of master mix was added to each diluted pre-selective amplification sample. The mix was vortexed briefly and centrifuged at 3,000rpm for 10 seconds. Selective amplification was carried out using Geneamp® PCR System 9700 (Applied Biosystems) under the conditions outlined in Table 5.6.

Table 5.6 Conditions of selective amplification

Cycles	Denaturing	Annealing	Extension	Soak
1	94°C – 2 min	65°C – 30 sec	72°C – 2 min	-
1	94°C – 1 sec	64°C – 30 sec	72°C – 2 min	-
1	94°C – 1 sec	63°C – 30 sec	72°C – 2 min	-
1	94°C – 1 sec	62°C – 30 sec	72°C – 2 min	-
1	94°C – 1 sec	61°C – 30 sec	72°C – 2 min	-
1	94°C – 1 sec	60°C – 30 sec	72°C – 2 min	-
1	94°C – 1 sec	59°C – 30 sec	72°C – 2 min	-
1	94°C – 1 sec	58°C – 30 sec	72°C – 2 min	-
1	94°C – 1 sec	57°C – 30 sec	72°C – 2 min	-
23	94°C – 1 sec	56°C – 30 sec	72°C – 2 min	-
Hold	-	-	60°C – 1 min	-
Hold	-	-	-	4 ° C ∞

There are 64 possible combinations of primers that can be used in the selective amplification reaction. Three primer pair combinations were used in this analysis and many primer combinations were tested prior to analysis (Table 5.7). Primers were selected for analysis on the basis of the quality and quantity of bands produced.

Table 5.7 Primer combinations tested prior to analysis

Combination	*Mse* I	*Eco*RI + Fluorescent marker	Dye Colour
1	-CTA	-ACA + FAM	Blue
2	-CAT	-ACT +FAM	Blue
3	-CAG	-ACT + FAM	Blue
4	-CAG	-ACG + JOE	Green
5	-CTG	-AAG + JOE	Green
6	***-CAT**	***-AGG + JOE**	**Green**
7	-CTG	-AGC + NED	Yellow
8	***-CTC**	***-AAC + NED**	**Yellow**

(*indicates combinations used in the data analysis, the other combinations were tested and not used)

Preparation of samples for automated AFLP analysis

Aliquots of primer amplified products were placed in 0.5 ml tubes with volumes varying depending on the fluorescent dye being used Table 5.8.

Table 5.8 Reaction volumes of selectively amplified DNA for AFLP analysis using an automated sequencer

Dye Colour	Primer Combination + Fluorescent Dye	Volume (per 0.5ml tube)
Blue = FAM	Cxx/Axx + FAM	0.6µl
Green = JOE	Cxx/Axx + JOE	0.8µl
Yellow = NED	Cxx/Axx + NED	1.3µl

24 µl of Foramide[19] and 0.3 µl of ROX 500s [20] size standard were added to each tube of primer amplified products. The samples were denatured at 95°C for 5 minutes and centrifuged at 3,000 rpm for 10 seconds and placed on ice until ready to load the machine. The tube lids were replaced with rubber septa prior to loading. Samples were then detected and visualised using an ABI prism 310 Genetic Analyser (Applied Biosystems) and GENESCAN® analysis software version 3.1 (Applied Biosystems) using the ROX 500s size standard module GS STR POP4 (1ml) F.

5.2.6 AFLP data handling and analysis

Data handling - Band Scoring

The AFLP fingerprint data were complied by comparing the presence or absence of peaks in the electropherogram (Figure 5.5) for each sample and scoring them as either 1 or 0, this was done using

[19] **Foramide:** CH_3NO – Applied Biosystems, used to denature the samples

[20] **Rox 500s:** Applied Biosystems – a fluorescently labelled size standard

GENOTYPER™ Software (Version 3.7 Applied Biosystems) (PE Applied Biosystems, 1996). Bands were scored visually at each base pair as either present or absent by inspection. Automatic scoring programmes are available but are considered unreliable, as peaks at one position, for example, 13 base pairs, can be digitally assessed as different, those at 13.1 base pairs, 13.3 base pairs etc. scored as separate loci. The length of each fragment in base pairs can be visualised on the X – axis and peak intensity on the Y-axis (Figure 5.5).

Fragments that differed from each other by more than 0.5 base pairs were identified as different. Peaks with intensity of less than 40 and above 350 were not scored and discarded as being unreliable, only bands clearly visible within the range were used in the analysis.

The final band/marker classifications obtained from manual checking produced a presence/absence matrix. Scoring error was calculated by scoring duplicate samples run in the same experiment conditions and comparing the scores. The data matrix obtained was inputted into Microsoft Excel.

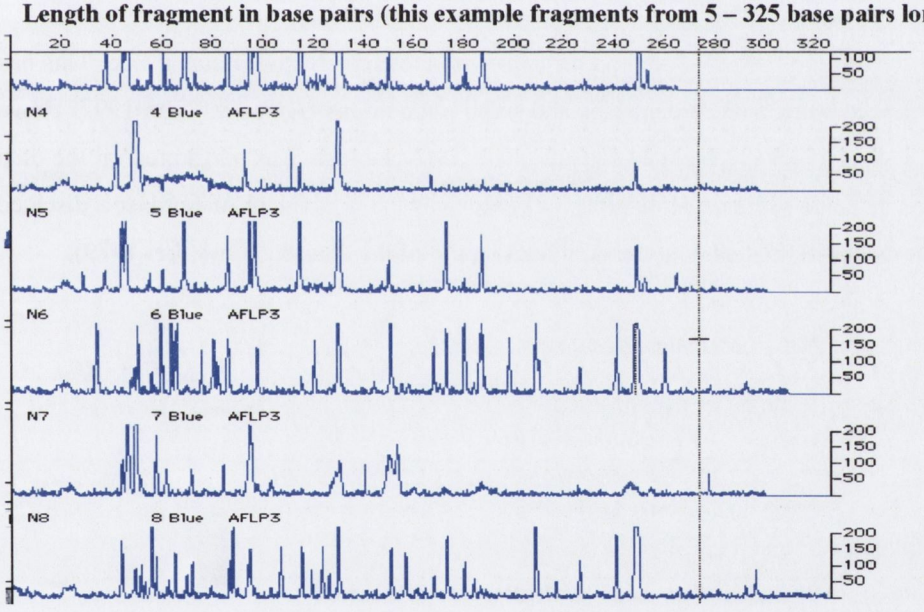


Figure 5.5 Electropherogram of AFLP bands and peaks using Genotyper ™ Software (Version 3.7 Applied Biosystems). The presence or absence of a peak (1 or 0) was noted for each sample and these data were entered into a Microsoft Excel spreadsheet for use in further analysis.

Cluster Analysis

UPGMA (unweighted pair-group with arithmetic mean) and Neighbour Joining were used to produce trees using distance matrices to show the relationship between different samples. UPGMA (Unweighted pair group with arithmetic mean) is a sequential clustering algorithm. It identifies two samples that are most similar to each other and treats them as a composite and compares other samples to the newly formed composite group. It continues to infer relationships in order of decreasing similarity and the tree is built in a stepwise manner (Swofford *et al.* 1996). Neighbour-joining is also based on finding two samples that are most similar (i.e. the genetic distance between the samples is short) clustering these together and comparing them to the other samples, the tree is then systematically built up (Li 1997). Neighbour-joining makes corrections for unequal rates of evolution between samples, which in this case is of little interest. There was no difference in the trees produced by either method in this case, UPGMA was chosen over neighbour joining for displaying results as it is deemed often the best method for recovering the "true tree" as demonstrated by computer simulation models (Nei 1987).

It is considered that the UPGMA method produces the most easily interpretable tree. This has been noted by authors who work with rare species and small populations (Ayres & Ryan 1997; Dowe *et al.* 1997; Stewart & Porter 1995; Krauss *et al.* 2002). UPGMA analysis was performed on the data using Nei-Lei genetic distance measure (Equation 5.2), which is the equivalent of Sørenson distance which was chosen for its ability to deal with the dominant nature of the dataset (Nei & Lei 1979).

$$\textit{Nei – Lei Genetic Distance} = 2a / (2a + b + c)$$

a is the number of polymorphic fragments that are shared in two samples
b is the number of fragments present in sample (i) and absent in sample (j)
c is the number of fragments present in sample (j) and absent in sample (i)

Equation 5.2 Nei – Lei Genetic Distance

Confidence levels of support for the robustness of the groupings and branch patterns produced by were assessed statistically using bootstrap. Bootstrapping tests the reproducibility of the results with re-sampling with replacement from within the data set. Bootstrap replicates with 1000 permutations were run and only groupings with a frequency greater than 50% are shown (Felsenstein 1985). The analyses were carried out in the software package PAUP 4 (Swofford 1999) [**P**hylogenetic **A**nalysis **U**sing **P**arsimony]. A genetic distance matrix was calculated in POPGENE Version 1.31 (Yeh *et al.* 1997) using Nei's (1978) unbiased estimate of genetic distances, which is deemed more suitable for

populations with small sample sizes. Ordinations were carried out using module S08, in PCA (principal coordinates analysis) Version 4.0 (Casgrain 1999).

5.2.7 Genetic diversity statistics

The extent of genetic diversity within the populations of *Coprosma benefica* and *Abutilon pitcairnense* on Pitcairn Island were assessed in POPGENE Version 1.31 (Yeh *et al.* 1997). The main consideration and advantage of using the POPGENE programme above others such as ARLEQUIN 2 (Sneider *et al.* 2000) is that diversity statistics can be calculated for populations not in Hardy-Weinberg (H-W) equilibrium.

Populations can only be considered to be in Hardy-Weinberg equilibrium if random mating maintains allele frequencies at constant levels from one generation to the next; this generally can only be considered the case in large out-breeding populations (Frankham *et al.* 2002). There is also no way to determine whether a population is in H-W equilibrium in the AFLP analysis of a single generation.

Given the fact that *Coprosma benefica* and *Abutilon pitcairnense* occur in extremely small numbers they are unlikely to be in Hardy-Weinberg equilibrium. In more recent times additional Bayesian statistical approaches have been developed (Holsinger *et al.* 2002; Zhivotovsky 1999; Krauss 2000) but research indicates that there is little difference between the traditional statistics and Bayesian models, though the Bayesian methods make less assumption about the data (Krauss 2000). It was decided to use traditional diversity measures as the statistics can be compared directly to other studies on small rare plant populations (Drummond *et al.* 2000; Zawko *et al.* 2001; Palacios *et al.* 1999; Godt & Hamrick 1999; Krauss 2002).

A quick estimate of diversity can be obtained from the percentage of polymorphic loci (Nei 1987). It generally is not presented in isolation as it is highly susceptible to sampling and scoring error. Shannon's Index of Genetic diversity I_S (Shannon & Weaver 1949) (Equation 5.3) is a more robust index commonly used to define both within and between population diversity (Lewontin 1973; King & Schaal 1989; Russel *et al.* 1993).

$$Is = -\sum pi \, Log_2 \, pi$$

pi is the frequency of a polymorphic loci

Equation 5.3 Shannon's Index of Genetic Diversity *Is*

Heterozygosity **h** is also considered a more robust measure of genetic diversity as it is determined in terms of gene frequency (Equation 5.4). Heterozygosity can only be inferred and not directly measured with dominant markers such as AFLP.

The heterozygosity for a single locus is given as:

$$h = 1 - \sum xi^2$$

where xi^2 is summed over 1 =1 to i=m

Equation 5.4 Heterozygosity *h*

Average heterozygosity, ***H***, is the average of h over all loci and describes the average proportion of heterozygotes per locus. The most widely used genetic diversity measures are Nei's (1972, 1973 & 1978) gene diversity statistics. They are also measures of heterozygosity but are derived from the probability calculations that two randomly chosen alleles from a population are different (Nei 1973). In these calculations the average gene diversity ***H*** (Equation 5.5) is estimated by sampling of the loci and is given by:

$$H = \frac{\sum_{J=1}^{r} hj}{r}$$

where r is the number of loci samples and hj is the value of h for the jth locus

Equation 5.5 Average proportion of heterozygosity per locus *H*

The total genetic diversity $\mathbf{H}_T$ (Equation 5.6) can be partitioned within H_S and between population diversity D_{ST}:

$$H_T = H_S + D_{ST}$$

Equation 5.6 Total Genetic Diversity H_T

Gene diversity can then be expressed as a proportion of the total population diversity to give G_{ST} (Equation 5.7):

$$G_{ST} = D_{ST} / H_T$$

Equation 5.7 Gene diversity G_{st}

The proportion of gene diversity within population can be calculated as $1 - G_{ST}$.

5.2.8 Sequencing of Chloroplast Gene

Target DNA Regions and Primer

The 800 base pair chloroplast *trn*L intron and *trn*L-F intergenic spacer (Taberlet *et al.* 1991) was amplified using primers C and F (MWG-Biotech) Table 5.9 These chloroplast DNA genes and region were chosen for the experiment based on a previously developed universal primer that is known to be reliable for amplification success over a range of species (Liu *et al.* 2005; Harbourne 2004; Murphy 2003; Ronsted *et al.* 2002; Hodkinson *et al.* 2002).

Table 5.9 Primer C and primer F base sequences

Primer	Primer Base Sequence
TRNLC	Forward primer C - 5'-CGA AAT CGG TAG ACG CTA CG-3'
TRNLF	Reverse primer f - 5'-ATT TGA ACT GGT GAC ACG AG – 3'

Protocol for cpDNA sequencing analysis

PCR Reaction for amplification of trnl-f gene region

PCR reactions were carried out with a volume of 50µl, using 0.5µl of primer at a concentration of 100ng/µl and 2µl of sample DNA. The amount of sample DNA used in the reaction can be varied depending on DNA concentration. With low concentrations the amount can be increased and with high concentrations the amount decreased. As all the samples used in this experiment had low concentrations of DNA (~ 250ng) 2µl of sample DNA was found to amplify well. A master mix with the components outlined in Table 5.10 was prepared. Mg^{2+} acts as a co-factor for *Taq* and catalyses primer binding, in experiments the amount of Mg^{2+} used was varied between 3- 4µl (increasing or decreasing the H_2O by 1µl to maintain a reaction volume of 50µl) depending on PCR amplification success.

Table 5.10 Components of PCR amplification reaction

Component	Volume per sample
Ultra pure H_2O	*36.75 µl
X 10 Buffer (Promega)	5.0 µl
Forward primer C	0.5 µl
Reverse primer F	0.5 µl
Mg^{2+}	*3.0 µl
Taq (Promega)	0.25 µl
	*increased or decreased depending on PCR amplification success

The PCR reaction mix of 38µl of Master Mix (Table 5.10) to 2µl of sample DNA were made up over ice and mixed thoroughly by vortex (Whirlimixer ™) and centrifuged at 3,000rpm for 10 seconds. The sample tubes containing master mix and sample DNA were loaded into a thermocycler

Geneamp® PCR System 9700 (Applied Biosystems) and run under the conditions outlined in Table 5.11

The annealing temperature was set at 48°C (2°C lower than general protocols) which was found to produce better amplification success, this maybe due to the fact that the sequencing primers (C and F) have a high G/C content (55 and 45 % respectively) and it is known that GC rich sequences cannot withstand high annealing temperatures.

Table 5.11 Reaction temperatures for PCR amplification

	Cycle				
	Each of 30 cycles				
Premelt	**Denaturing**	**Annealing**	**Extension**	**Final Extension**	**Soak**
95°C	95°C	48°C	72°C	72°C	4°C
1minute	45 seconds	45 seconds	3 minutes	7 minutes	∞

The amplification success was verified using agarose gel electrophoresis (using 4µl of PCR product to 1µl of loading dye) as outlined in section 4.2.4. A successful amplification produces narrow bands of product of the same length (Figure 5.6).

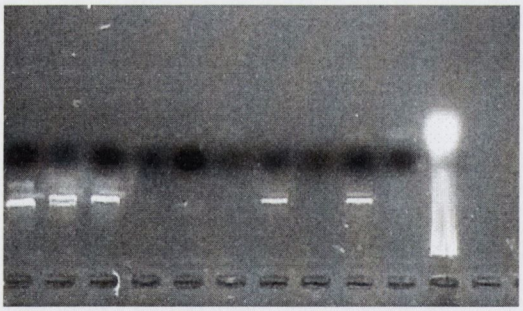


Figure 5.6 Fluorescent narrow bands of successfully PCR amplified samples In this case samples(read from left) in wells 1,2,3,7 and 9 were successfully amplified. The sample in well 11 is total DNA .

The PCR products were cleaned using JETQUICK™ PCR purification Spin Kit (GENOMED) which involves binding, washing and eluting the PCR amplified DNA.400µl of Binding solution (H1)[21] was added to 50µl of PCR product, mixed with a pipette, transferred to a JETQUICK spin column in a 2ml receiver tube and centrifuged for 1 minute at 13,000rpm. The flow through was discarded. 500ul of

[21] **H1:** concentrated guanidine hydrochloride, EDTA, Tris/HCL and isopropanol

wash buffer (H2)[22] was added to the JETQUICK spin column and further centrifuged for 1 minute at 13,000 rpm and the flow through was again discarded.

A final centrifuge at 13,000rpm for 1 minute was applied to the samples to get rid of any remaining wash buffer. The PCR-amplified DNA was then eluted using 50µl of ultra pure sterile water which was preheated to 65°C and applied to the centre of the silica membrane in the JETQUICK spin column. The JETQUICK spin column was placed in a new 1.5ml tube and allowed to stand for 3 minutes. A final centrifuge at 13,000rpm for 2 minutes was then applied and the cleaned PCR-amplified DNA was stored at -20°C.

PCR Amplification Cycle sequencing of chloroplast gene region trnl-f

The cleaned amplified PCR products were then prepared for sequencing by aliquotting 3µl into flat-topped 0.5 ml tubes. A master mix containing Applied Biosystems *Taq* Dye-deoxy/terminator cycle sequencing mix V.1.1 (PINK MIX[23]), sequencing buffer [24] sterile water and sequencing forward and reverse primers c & f (separate master mixes for each primer) to a total volume of 10µl was made up with the components of Table 5.12. Components and mixes were made up over ice.

Table 5.12 Components of Cycle sequencing reaction.

Component	Volume per sample
Ultra pure H_2O	1.8µl
Pink mix	1µl
Sequencing buffer	3.5µl
Cleaned PCR product	3µl
Primer c (forward primer) or Primer f (reverse primer)	0.7 µl

Samples were mixed thoroughly by vortex and centrifuged at 3,000rpm for 10 seconds and loaded onto the thermocycler Geneamp® PCR System 9700 (Applied Biosystems) which was set at the reaction conditions outlined in Table 5.13.

[22] **H2:** ethanol, NaCl, EDTA and Tris/HCL

[23] **Pink Mix:** Big Dye™ Terminator V1.1 Cycle sequencing RR-100 containing Big Dye terminators which stop synthesis of DNA and has a fluorescent dye attached it also contains DNTp's spare bases for DNA synthesis and polymerase.

[24] **Sequencing Buffer:** 200mM Tris-HCL, 5mM $MgCl_2$ (pH 9.0)

Table 5.13 Conditions for amplification of forward and reverse primer sequences of the chloroplast trnl-f region

Cycle		
28 cycles		
96°C	50°C	60°C
10 seconds	5 seconds	4 minutes

Sample purification and cleaning prior to sequencing

The forward and reverse amplified primer samples were removed from the thermocycler and cleaned using an alcohol wash and precipitation method prior to running. Flat topped 0.5ml tubes were labelled and all the amplified cycle sequenced product added. To each sample 50µl of ethanol (EtOH:100%) and 2µl of Sodium acetate (NaOAc: 3M) was added and samples incubated at room temperature for 5 minutes, and further incubated on ice for 10 minutes after which they were centrifuged at 13,000rpm. The samples were then drained on clean tissue to remove the ethanol (Cycle sequenced DNA product pellets at the bottom of the tube). Two further ethanol washes and tissue draining with 300µl (EtOH: 70%) were carried out on each of the samples and centrifuged at 13,000rpm for 15 minutes each time. The drained tubes were put between layers of tissue to exclude light which can degrade the DNA to ensure any of the remaining ethanol has evaporated off.

Preparation of samples for sequencing

The cleaned and dried samples were prepared for long run sequencing (55 minutes) by adding 25µl of TSR[25] into each tube. The samples were then mixed thoroughly by vortex and incubated to denature at 95°C for 5 minutes. Samples were then placed in ice for 3 minutes and centrifuged at 3,000rpm for one minute. The flat lids of the tubes were then removed and rubber septa inserted into each tube. Tubes containing the sequenced samples were loaded onto an ABI PRISM 310 Genetic Analyser (Applied Biosystems). The machine setting used were ABI PRISM™ 310 module, Seq. Pop6 Rapid (1.0ml) E using Pop 6 polymer for 55 minutes per sample. The sequence data from each sample was automatically saved and processed in ABI PRISM ™ Sequence Analysis Version 3.4.1 (Applied Biosystems).

5.2.8 Sequence Data Analysis

Successful samples were obtained were edited and assembled in the software package SEQUENCHER (© Gene Codes Corporation, 1998) Version 3.1. Forward C and reverse F sequences were joined where complementary bases overlapped and a Contig was formed i.e. a complete gene sequence, in this case

[25] **TSR:** Sample preparation reagent, Template Suppression Reagent

the 800 base pair long *trnl-f* intron and *trnl-f* intergeneric spacer. The completed *Contig* sequence was produced for all samples.

Samples were aligned visually using PAUP 4 (Swofford 1999) as all samples were of the same gene region. This was used to form a matrix of bases by sample. Sequences from GENBANK (NCBI database) were included in the data set and aligned in PAUP 4 (Swofford 1999)

5.3 Results

5.3.1 Lastreopsis c.f. pacifica

Population Survey

Lastreopsis c.f. *pacifica* was described as a new record for Pitcairn Island in 1997 and classified as critically endangered (Kingston & Waldren 2005) it occurs in two populations at Brown's water and Pulau Bridge on the island. It is native in Polynesia in Samoa, the Cook Islands and the Society Islands. The main population at Brown's Water is highly threatened due to road expansion schemes and the threat of erosion. It occurs in woodland dominated by *Syzygium jambos* and is found growing in association with *Trichomanes endlicherianum* on volcanic tuff (Plate 6).

The total area covered by the main population was 20 x 60m (Kingston 2001) and this population had not expanded in area in 2003, 2004 & 2005. The second population which consisted of a sole sterile individual in 1997 had expanded to six mature individuals and two juveniles found in an area of 10 x 5m in 2003, 2004 & 2005. The following size classes (Table 5.14) for census were devised based on the number of fronds and the height and width of the individuals assessed. One plant from each population was taken to the nursery and grown on for two years. Two plants were obtained by division of the nursery plants, one from each population. These were subsequently planted at a *in-situ* conservation site developed at Big Rock, Pulau in 2005.

Table 5.14 Population size classes used in census of *Lastreopsis* c.f. *pacifica*

Population size classes of *Lastreopsis* c.f. *pacifica*				
		Juvenile	**Mid-sized**	**Mature**
No. of Fronds	**Mean± St. Dev**	2.92±1.36	3.49±1.56	4.87±1.59
Height	**Mean± St. Dev**	6.61±3.42cm	14.95±2.31cm	27.12±7.55cm
Width	**Mean± St. Dev**	7.89±4.08cm	14.84±4.19cm	28.68±7.70cm

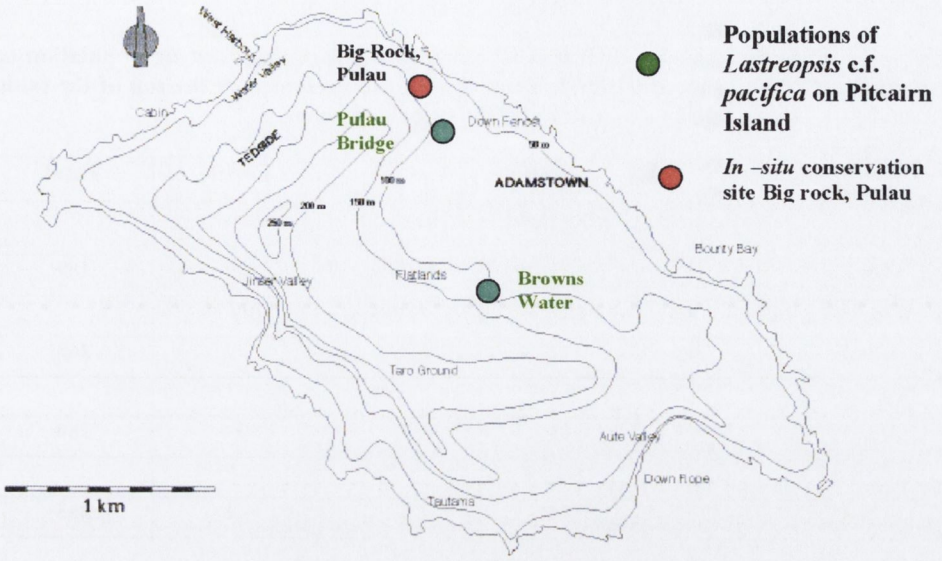


Plate 6 *Lastreopsis* c.f. *pacifica* population locations and habit

The numbers of juvenile, mid-sized, mature and mature fertile individuals in each population at the time of census are outlined in Table 5.16 and graphically arranged for each population location in Figures 5.7a and b. The percentage totals of mature fertile plants have increased steadily since 1997 (10.6%, 11.1%, 13.9% & 14.4%).

While there was an initial percentage total rise in the number of juvenile individuals recorded from 1997 to 2003 (25.9% to 27.5%) there has been little change in the number of juvenile individuals since 2003 (27.5%, 27.5% & 27.0%). Mid-sized individuals decreased from 1997 to 2003 (48.1% to 30.48%) and then remained steady (30.5%, 30.4, & 30.2%) and the percentage total of mature plants almost doubled between 1997 and 2003 (15.3% to 30.8%) then decreased 2 percentage points during 2003/2004 (28.2%) and 2004/ 2005 (28.4%) (Table 5.16).

Table 5.16 Distribution and numbers of individuals of *Lastreopsis* c.f. *pacifica* during population census years 1997, 2003, 2004 & 2005 on Pitcairn Island. Total figures are presented at the top of the table and figures for individual population at Brown's Water and Pulau Bridge below.

		Sterile			Fertile	Total
Year		**Juvenile**	**Mid-sized**	**Mature**	**Mature**	
1997	**Total**	49	91	29	20	**189**
1997	*% of Total*	*25.9%*	*48.1%*	*15.3%*	*10.6%*	
2003	**Total**	74	82	83	30	**269**
2003	***% of Total***	*27.5%*	*30.5%*	*30.8%*	*11.1%*	
2004	**Total**	77	85	79	39	**280**
2004	*% of Total*	*27.5%*	*30.4%*	*28.2%*	*13.9%*	
2005	**Total**	77	86	81	41	**285**
2005	*% of Total*	*27.0%*	*30.2%*	*28.4%*	*14.4%*	
	Location					
1997	**Brown's Water**	49	91	28	20	188
2003	**Brown's Water**	72	82	79	28	261
2004	**Brown's Water**	75	84	77	35	271
2005	**Brown's Water**	75	85	79	37	276
	Location					
1997	**Pulau Bridge**	0	0	1	0	1
2003	**Pulau Bridge**	2	0	4	2	8
2004	**Pulau Bridge**	2	1	2	4	9
2005	**Pulau Bridge**	2	1	2	4	9

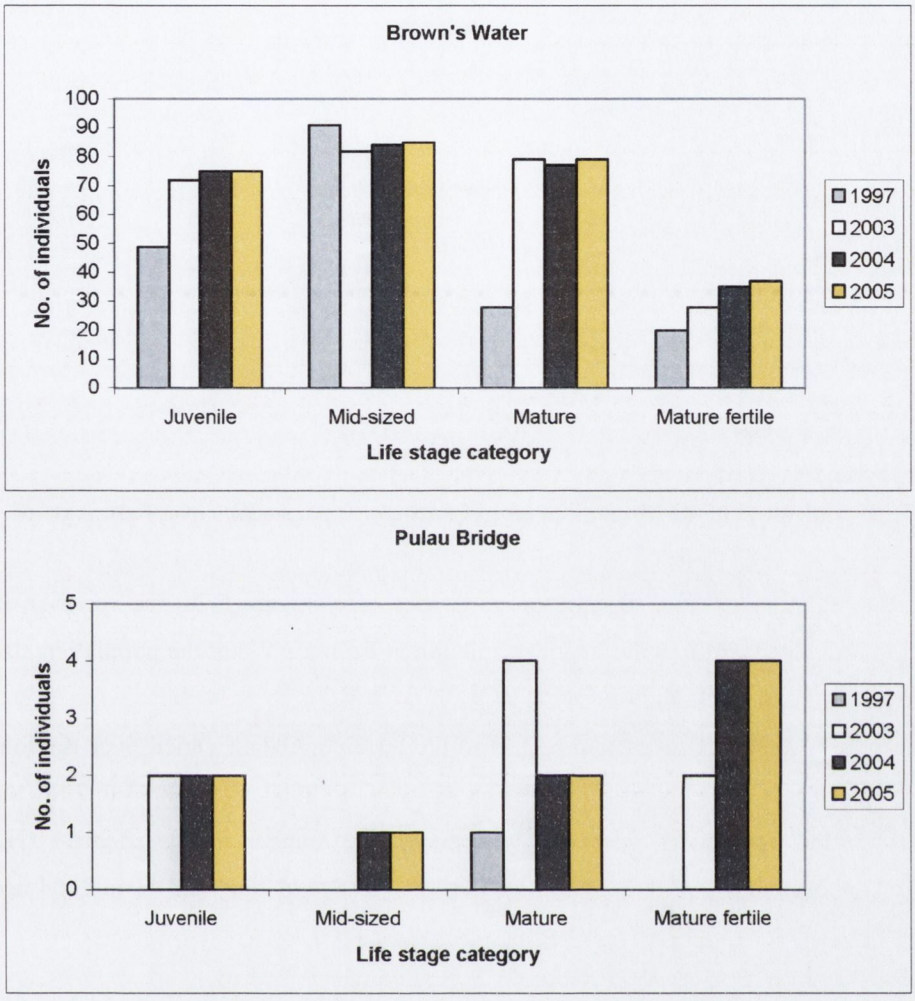


Figure 5.7 Population census of *Lastreopsis* c.f. *pacifica* at Brown's Water and Pulau Bridge

The two populations have increased in size since the start of the study (Figure 5.7). The changes in population size can be expressed as the growth rate, λ (Equation 5.1). A growth rate of 1 indicates the steady state where population is not increasing or decreasing. The growth rate varied from 8 to 1 in the Pulau Bridge population and decreased from 1.388 to 1.018 in the Brown's Water population (Figure 5.8).

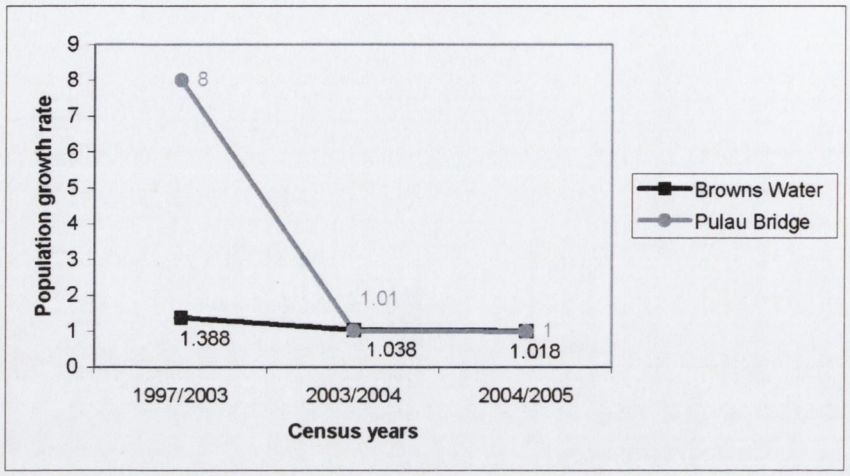


Figure 5.8 Variation in growth rate λ, in the two populations of *Lastreopsis* c.f. *pacifica* over four years of census monitoring.

In the extremely small population at Pulau Bridge the population size (Figure 5.7b) the variation in growth rate (Figure 5.8) fluctuated while in the larger population at Brown's Water the population size (Figure 5.7 a) and growth rate was stable Figure 5.8).

The total variation in the numbers of individuals in the total population varied over the four census years with the biggest increase between the census years 1997 to 2003. From 2003 to 2005 the number of individuals in the population increased by sixteen. The number in the effective (N_e) population also rose by ten between 1997 and 2003, nine between 2003 and 2004 and 11 in 2004 and 2005 (Figure 5.9).

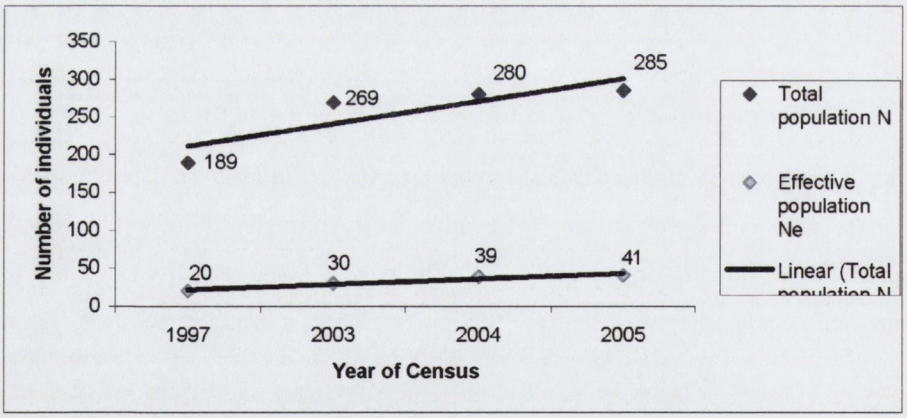


Figure 5.9 (above)Variation in the total population and effective population of *Lastreopsis* c.f. *pacifica* during the four census years

Table 5.17 A summary of the current site and conservation information for *Lastreopsis* c.f *pacifica*

Site Description		
	Brown's water	**Pulau bridge**
GPS Location	S 25°04.139' W 130° 06. 273'	S 25°03.899' W 130°06.295'
Altitude	250m	88m
Slope	60°	15°
Aspect	NW	NW
Landform	On steep rock face	On rocks beside stream
Vegetation	*Syzygium jambos* invaded woodland	*Syzygium jambos* invaded woodland
Land tenure	Public stream	Unknown
Management		
Quality	1	4
Condition	2	3
Long term prospects	3	2
Site protection	4	2
Overall Score	**10**	**11**
Propagation method	Division & Spore germination	Division & Spore germination
Conservation collections (in-situ & ex-situ)	Pitcairn Island Nursery, Big Rock, Pulau, Trinity College Botanic Gardens	Pitcairn Island Nursery, Big Rock, Pulau Trinity College Botanic Gardens

Quality - consider the size and productivity of the population and the vigour of individuals. (**1**= >100 individuals; **2**=51-99 individuals; **3**=<50 individuals; **4**=<10 individuals)
Condition - Habitat pristine or degraded (**1**=excellent; **2**=good; **3**=marginal; **4**=poor).
Long term prospects-The long term prospects for continued existence of this population at the indicated site. (**1**=excellent; **2**=good; **3**=marginal; **4**=poor.)
Site protection – Population protected from extrinsic human factors (**1**=excellent; **2**=good; **3**=marginal; **4**=poor).
Overall Score - a summary of all above listed factors with summary value (**4**=excellent; **8**=good; **12**=marginal; **16**=poor)

5.3.2 *Haloragis* sp.

Population census

Haloragis sp. was first recorded for Pitcairn Island in 1997 (Kingston 2001). It is a small woody shrub in the Haloragaceae, it is recorded to be locally distributed and restricted to southern coastal regions of the Island (Kingston 2001). A survey of three of the five known locations was carried out in 2003 and a population census was carried out at these locations (Plate 7). One plant from each population was brought into cultivation in the Island nursery in 2003.

Eight-hundred and eight-eight individuals of *Haloragis* sp. were found occurring in the three populations. The main population centre at Arliehow Ridge had 758 individuals or 85.4% of the total population surveyed, the site at Tautama had 85 individuals or 9.6% of the population surveyed and the site at Down Rope has 45 individuals or 5.0% of the population surveyed. The effective population sizes N_e of the three populations are much lower than the census count, with 161 flowering adults at Arliehow ridge, 15 at Tautama and 5 at Down Rope (Figure 5.8).

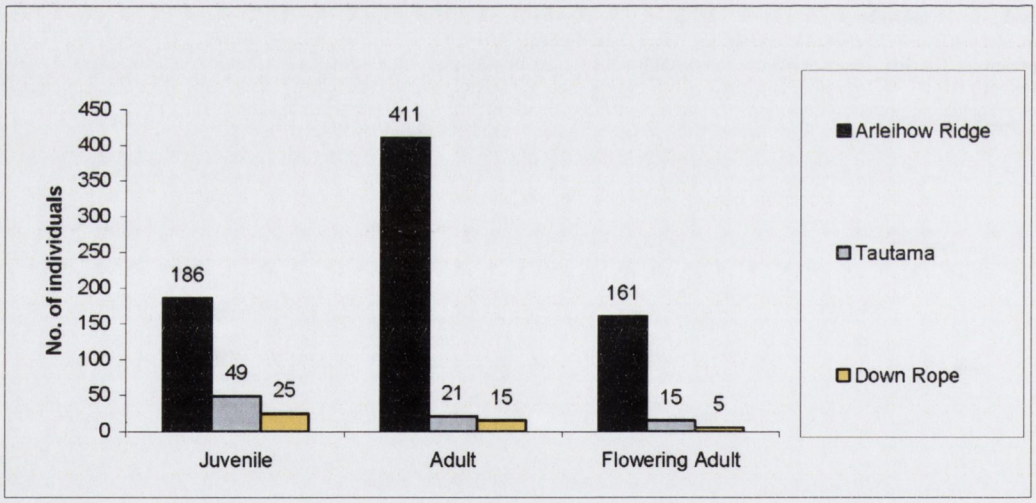


Figure 5.8 Population census of *Haloragis* sp. in 2003 at three of the five known population locations on Pitcairn Island.

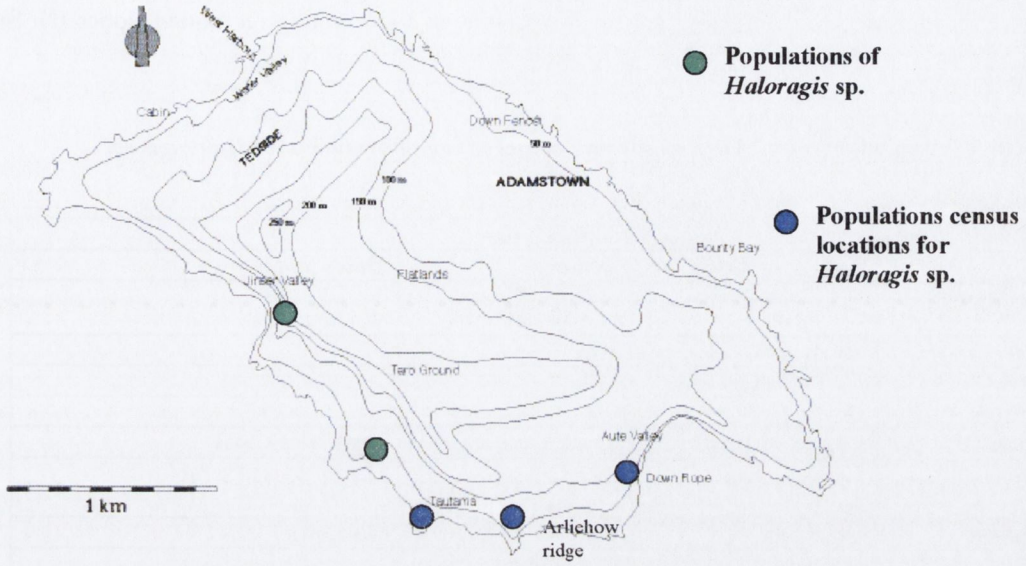


Plate 7 ***Haloragis*** **sp. locations and habit**

A conservation appraisal for *Haloragis* sp. is outlined in Table 5.18. The population at Arliehow ridge appeared to be larger than the population discovered in 1997 (S. Waldren *pers. comm.*) which may indicate that the population is expanding along the southern cliffs of the island. The mean overall score for the sites is 8.6, which is good; while the habitat itself is prone to erosion, areas which were recently

eroded were vegetated with *Haloragis* sp. so it is capable of regenerating on eroded slopes (N. Smyth *pers. obs.*).

Table 5.18 A full summary of the current site and conservation information for *Haloragis sp.*

Site Description			
	Arliehow Ridge	**Tautama**	**Down Rope**
GPS Location	S 25°04.757' W 130° 06. 135'	S 25°04.668' W 130°06.181'	S 25°04.899' W 130°06.101'
Altitude	250m	107m	80m
Slope	60°	65°	70°
Aspect	S	S	S
Landform	On steep clay bank	On steep clay bank	On steep clay bank
Vegetation	Grassy coastal scrub	Grassy coastal scrub	Grassy coastal scrub
Land tenure	Unknown	Unknown	Unknown
Management			
Quality	1	2	3
Condition	3	3	3
Long term prospects	2	2	2
Site protection	2	2	2
Overall Score	**8**	**9**	**9**
Propagation method	Division and cuttings	Division and cuttings	Division and cuttings
Conservation collections (In-situ & ex-situ)	Pitcairn Island Nursery	Pitcairn Island Nursery	Pitcairn Island Nursery

Quality - consider the size and productivity of the population and the vigour of individuals. (**1**= >100 individuals; **2**=51-99 individuals; **3**=<50 individuals; **4**=<10 individuals)
Condition - Habitat pristine or degraded (**1**=excellent; **2**=good; **3**=marginal; **4**=poor).
Long term prospects -The long term prospects for continued existence of this population at the indicated site. (**1**=excellent; **2**=good; **3**=marginal; **4**=poor.)
Site protection - Population protected from extrinsic human factors (**1**=excellent; **2**=good; **3**=marginal; **4**=poor).
Overall Score - a summary of all above listed factors with summary value (**4**=excellent; **8**=good; **12**=marginal; **16**=poor)

5.3.3 *Myrsine* aff. *niauensis*

Sole individual and location details

Myrsine aff. *niauensis* was refound in 2003 by Dr. S. Waldren (Plate 8) it had been presumed to be extinct on Pitcairn Island as no records exist of it since the late 19th century (collected in 1898 by islander R. Young and in 1830 by A. Matthews) and it was not found during the flora and vegetation survey of 1997 (Kingston 2001).

A full survey of the location and conservation assessment was prepared (Table 5.19). The plant is in a very precarious location beside a road verge, grading and clearing of road verge vegetation in 2005 very nearly uprooted it. Cuttings were taken in 2003, 2004 and 2005 and plants obtained are in cultivation in a few ex-situ locations (Table 5.19). Drawings were prepared of *Myrsine* aff. *niauensis* (Plate 9) .

Table 5.19 A full summary of the current site and conservation information for *Myrsine aff. niauensis.*

Site Description	
	McCoy's valley Road
GPS Location	S 25°04.518' W130°06.230'S
Altitude	210m
Slope	10°
Aspect	SE
Landform	On clay bank beside McCoy's Valley road
Vegetation	Fragmented *Homalium taypau* & *Meterosideros collina* woodland invaded with *Lantana camara*
Land tenure	Unknown
Management	
Quality	4
Condition	4
Long term prospects	4
Site protection	4
Overall Score	**16**
Propagation method	Cuttings
Conservation collections (In-situ & ex-situ)	Pitcairn Island Nursery, Trinity College Botanic Gardens and National Botanic Gardens, Glasnevin
Notes	Flowering season on Pitcairn Island : May-June - Male flowers

Quality - consider the size and productivity of the population and the vigour of individuals. (**1**= >100 individuals; **2**=51-99 individuals; **3**=<50 individuals; **4**=<10 individuals)
Condition - Habitat pristine or degraded (**1**=excellent; **2**=good; **3**=marginal; **4**=poor).
Long term prospects-The long term prospects for continued existence of this population at the indicated site. (**1**=excellent; **2**=good; **3**=marginal; **4**=poor.)
Site protection – Population protected from extrinsic human factors (**1**=excellent; **2**=good; **3**=marginal; **4**=poor).
Overall Score - a summary of all above listed factors with summary value (**4**=excellent; **8**=good; **12**=marginal; **16**=poor)

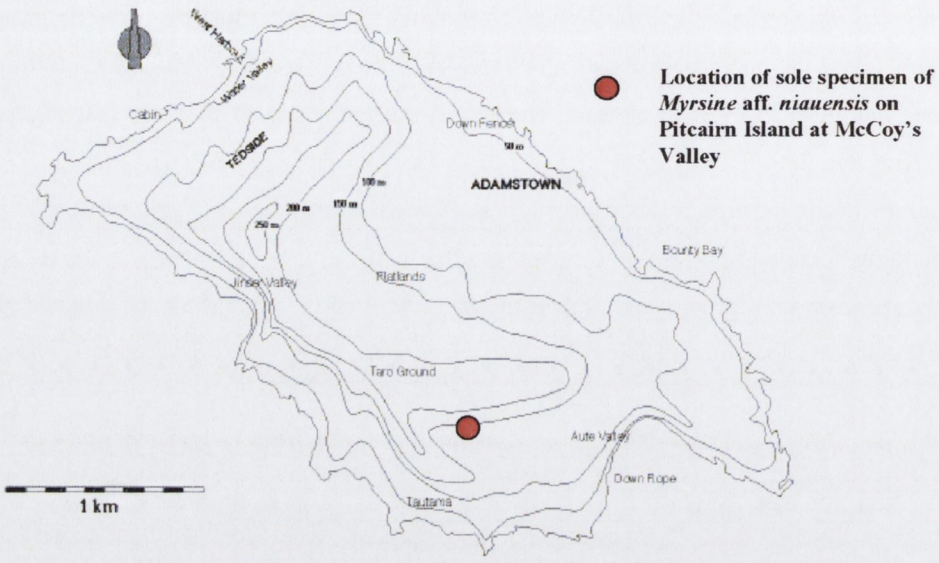
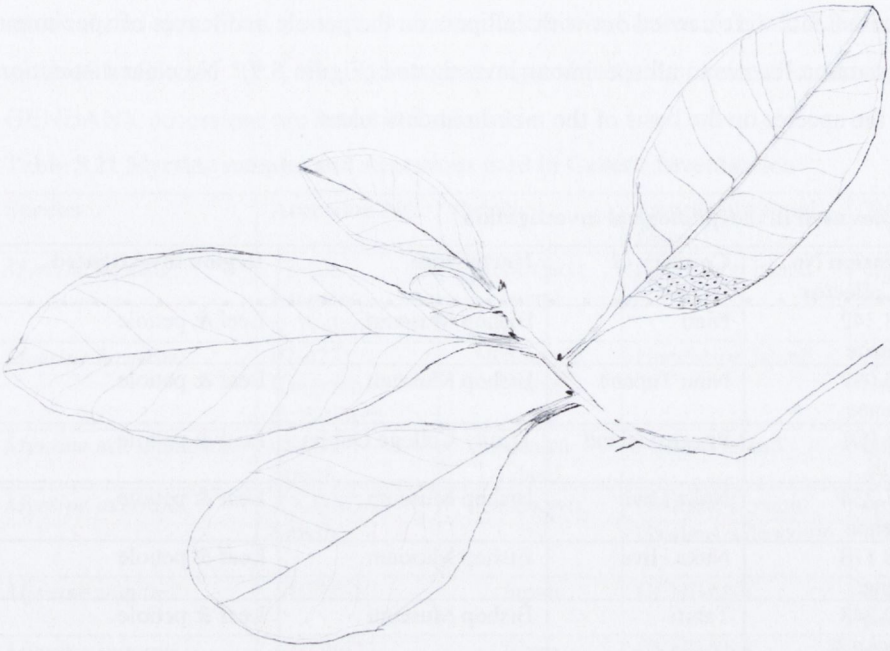


Plate 8 Sole location and habit of *Myrsine* aff. *niauensis* on Pitcairn Island



a. Actual size drawn by Smyth, N.

b. Habit in the wild

Plate 9 *Myrsine* aff. *niauensis* sole plant habitat (b) and drawing (a)

Morphological Analysis

Herbarium specimens were obtained from the Bernice P. Bishop Museum in Hawaii for morphological investigations and comparison (Table 5.20) to determine the specific identity of *Myrsine* aff. *niauensis*

on Pitcairn Island. Measurements were carried out with callipers on the petiole and leaves of specimens as these were the only common feature to all specimens investigated (Figure 5.9). No clear distinction could be made between the species on the basis of the measurements taken.

Table 5.20 Myrsine samples used in morphological investigation

Species	Accession No. & Collector	Country of Origin	Herbarium	Region investigated
Myrsine niauensis	1993.342 Florence	Niau	Bishop Museum	Leaf & petiole
Myrsine niauensis	1995.097 Florence	Niau Tupana	Bishop Museum	Leaf & petiole
Myrsine aff. niauensis	2005.001 Smyth	Pitcairn Island	Trinity College Dublin	Leaf & petiole
Myrsine adamsonii	1997.234 Florence	Nuka Hiva	Bishop Museum	Leaf & petiole
Myrsine adamsonii	1998.178 Wagner	Nuka Hiva	Bishop Museum	Leaf & petiole
Rapanea falcata	1980.243 Teraoka & Kennedy	Tahiti	Bishop Museum	Leaf & petiole
Myrsine hosakae	1997.429 Waldren &Kingston	Henderson Island	Trinity College Dublin	Leaf & petiole

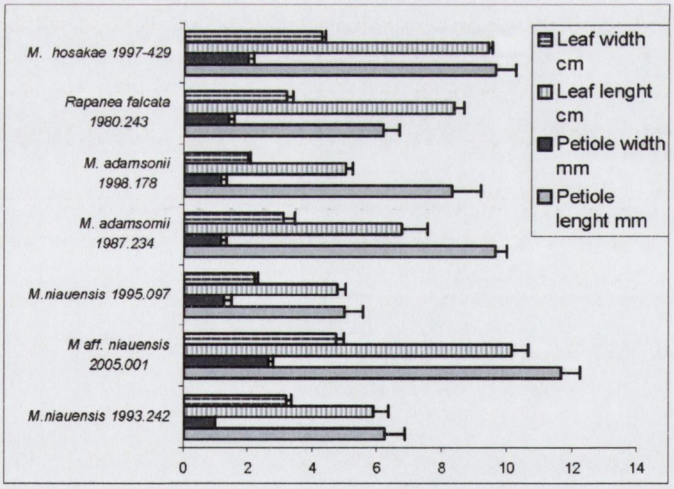


Figure 5.9 Mean leaf and petiole measurements (+SE) on *Myrsine* species examined (n=10) for each measurement

Genetic Analysis

Samples for genetic analysis were obtained from the sole individual on Pitcairn Island and *Myrsine hosakae* from Henderson Island (Kingston & Waldren: 1997.426 & 1997.426). Fresh material was collected from *Myrsine australis* at the National Botanic Gardens, Glasnevin and other *trn*L-F sequences of *Myrsine* species were obtained from GENBANK. DNA was extracted from the leaf

material and quantified using techniques outlined in Section 5.2.3 & 5.2.4 and sequencing reactions outlined in Section 5.2.8. The species investigated with their scientific names, geographic origins and GENBANK accessions are listed in Table 5.21

Table 5.21 Myrsine samples and Accessions used in Genetic Investigation

Species	Accession No.	Origin	Sample obtained from	Target region
Myrsine hosakae	97-426 Kingston & Waldren	Henderson Island	Henderson Island	cpDNA *trn*L-F
Myrsine hosakae	97-427 Kingston & Waldren	Henderson Island	Henderson Island	cpDNA *trn*L-F
Myrsine aff. *niauensis*	05-001 Smyth	Pitcairn Island	Pitcairn Island	cpDNA *trn*L-F
Myrsine australis	XX.008765 Smyth	Unknown	National Botanic Gardens, Glasnevin	cpDNA *trn*L-F
Myrsine seguinii	ABI78629	Japan	GENBANK	cpDNA *trn*L-F
Myrsine seguinii	ABI78627	Japan	GENBANK	cpDNA *trn*L-F
Myrsine faberi	AF547797	China	GENBANK	cpDNA *trn*L-F

The sequences obtained were viewed in SEQUENCHER software. The variation in the *trn*L-F region revealed that *M. hosakae* and *M.* aff. *niauensis* shared a number of nucleotide substitutions not found in the other sequences examined but their sequences were not identical (Appendix CD). Analysis using a exhaustive parsimony search for possible trees was carried out in PAUP 4.0, samples were equally weighted, stepwise addition of branches was selected and 1000 random replicates chosen with the swapping algorithm TBR. The parsimony search resulted one tree with a length of 1194, and with 650 parsimony informative characters, the consistency index (CI) was 0.90 and retention index (RI) 0.75 (Figure 5.10) Bootstrap analysis with 1000 replicates assigned strong support for the relationships (the bootstrap values are presented Figure 5.10). The cladogram was arranged with *Myrsine* species as an un-rooted monophyletic group. The species of interest *M.* aff *niauensis* grouped with *M. hosakae* of Henderson Island, as a sister group to the rest of the *Myrsine* species analysed. The proportion of nucleotide sites differing between *M.* aff. *niauensis* and *M. hosakae* was 0.0072; *M.* aff. *niauensis* and M. *seguinii* 0.7774; *M.* aff. *niauensis and M. australis* 0.7085 and *M.* aff. *niauensis* and M. *faberi* 0.7813. These results indicate that the closest relatives of the *Myrsine* aff. *niauensis* are not surprisingly in the Pacific at Henderson Island and Japan and despite being very closely related to *M. hosakae,* M. aff. *niauensis* on Pitcairn Island is still marginally distinct from it.

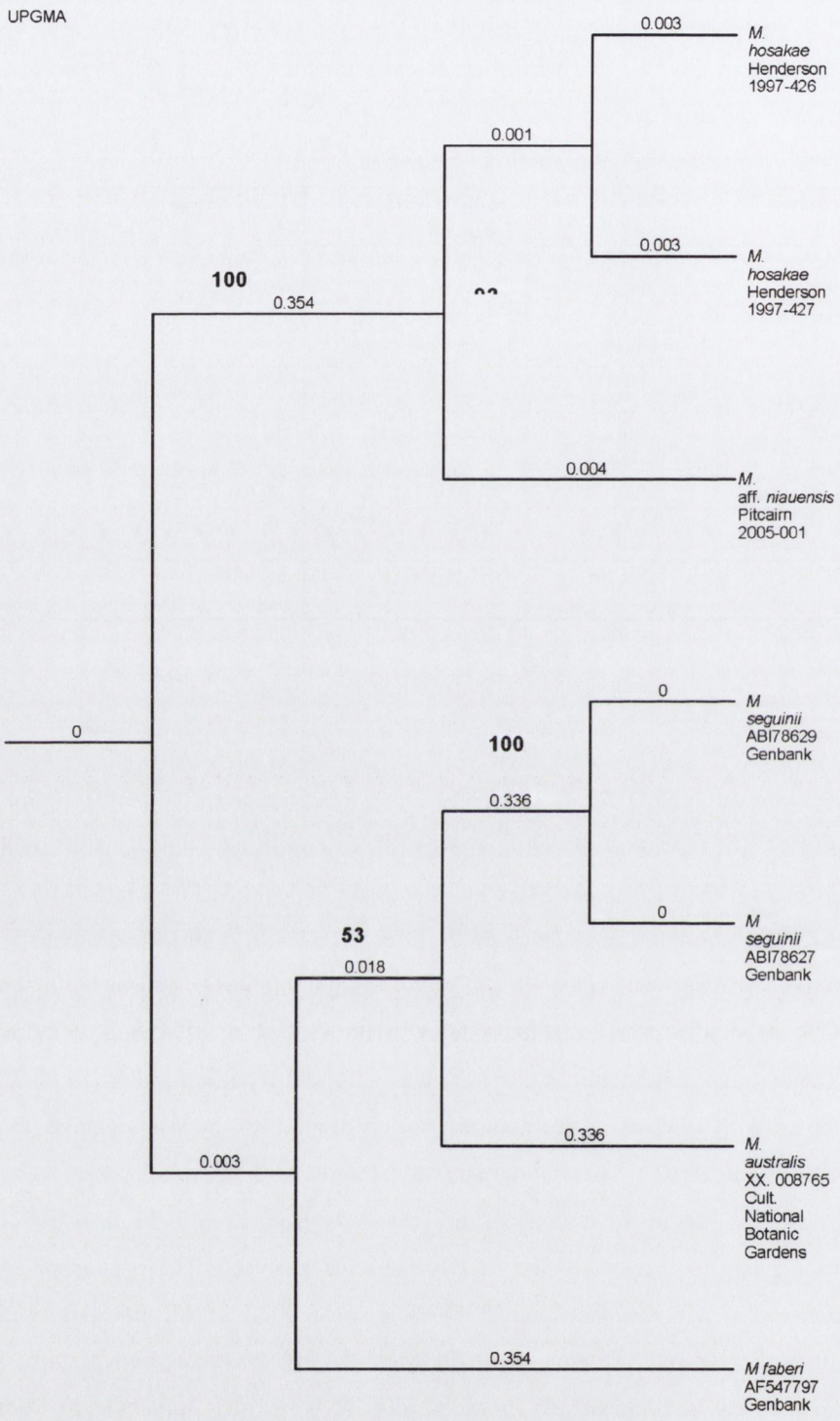


Figure 5.10 The single most parsimonious cladogram for *Myrsine* sp. examined species based on trnL-F data. Numbers above the branches indicate branch lengths. Values in bold indicate bootstrap support greater than 50% for individual clades.

5.3.3 Abutilon pitcairnense

Population census

Abutilon pitcairnense was refound in 2001 by Carol Warren (Plate 10 and 11) and been presumed extinct on Pitcairn Island as no records or sightings were made of it since the mid 20th century. It last seen in 1955 by I.T. Twyford and last collected by H. St. John in 1934 and it was not found during the flora and vegetation survey of 1997, despite detailed searches of known locations (Kingston 2001).

Carol Warren took some cuttings from the sole wild newly found individual at Tedside in 2001 (Plate 5.6). She planted one of the rooted cuttings at Flatlands during 2002 and it was seen to be growing very well during fieldwork in 2003. The plant at Flatlands had two flowers which lasted from the 7th to the 14th July 2003 (Plate 5.5). Cuttings and seed were obtained from this clone at Flatlands in July and August 2003. In 2004, seven seedlings had germinated and survived (Seedling no. 3, 4, 5, 6, 7, 9, and 10) from the fifty seed sown and three of the seedlings (seedling 4, 5 and 9) were then planted beside the original Tedside plant in 2004. A subsequent landslide destroyed the original Tedside plant and three planted seedlings at Tedside also in January 2005. Rooted cutting material was brought to Trinity College Botanic Gardens in August 2003 and the plant obtained flowered during-winter on an annual basis. However, hand-pollination experimentation at the gardens has not resulted in seed set. In 2005 a new *in-situ* conservation site at Big Rock by Pulau was developed on Pitcairn Island plants obtained from cutting material and seedling 6 was planted at this location, this area is considered secure from landslip and is regularly visited by the conservation officer. Cuttings root easily and many clones of *Abutilon pitcairnense* are now growing in the island nursery along with seedlings 3, 7 and 10. Details of the sites and locations where *Abutilon pitcairnense* were formerly found and are currently found are outlined in Table 5.22.

Table 5.22 A summary of the current sites and conservation assessment for *Abutilon pitcairnense*

Site Description			
	Tedside Original & seedlings 4,5, & 9	**Flatlands Clone**	**Big Rock, Pulau Clone & Seedling 6**
GPS Location	S 25°04.106' W 130° 06. 435'	S 25°04.088' W 130°06.420'	S 25°04.910' W 130°06.312'
Altitude	202m	199m	88m
Slope	80°	0°	10°
Aspect	W	NW	SE
Landform	Steep clay bank	Flat road verge	Open clearing
Vegetation	Degraded *Homalium taypau and Pandanus tectorius* woodland	Near former *Syzygium jambos* forest	Near *Pandanus tectorius* woodland
Land tenure	Unknown	Unknown	Unknown
Management			
Quality	Extinct	4	4
Condition	Extinct	3	2
Long term prospects	Extinct	3	2
Site protection	Extinct	4	2
Overall Score	Extinct	**14**	**10**
Propagation method	Cuttings	Cuttings and Seed	Cuttings
Conservation collections (In-situ & ex-situ)	Pitcairn Island Nursery and Trinity College Botanic Gardens	Pitcairn Island Nursery: cuttings and seedlings 3, 7 &10	Pitcairn Island Nursery cuttings and cuttings of seedling 6
Notes	Tedside site lost in a landslide in January 2005	Growing well in 2003, 2004, 2005 & 2006	Reported to be growing well in 2006

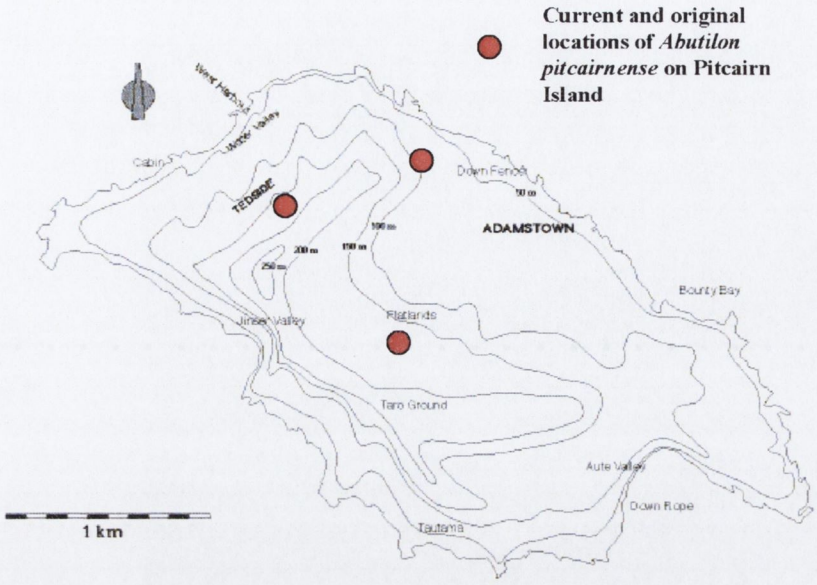


Plate 10 Current and original locations (top) and flower of *Abutilon pitcairnense* on Pitcairn Island

Plate 11 ***Abutilon pitcairnense*** **in the original site at Tedside before it was destroyed in a landslide in January 2005.**

Genetic Analysis

Samples for genetic analysis were obtained from the wild individual at Tedside, the clone from the wild individual at Flatlands and seedlings obtained from the Flatland clone in 2003. DNA was extracted from the leaf material and quantified using techniques outlined in Section 5.2.3 & 5.2.4 and AFLP reactions outlined in Section 5.2.5. The full lists of samples used are listed in Table 5.23.

Table 5.23 *Abutilon pitcairnense* samples analysed

Sample	Accession No.	Current Status on Pitcairn Island
Seedling 3	2005.003	Live
Seedling 4	2005.004	Dead
Seedling 5	2005.005	Dead
Seedling 6	2005.006	Live
Seedling 7	2005.007	Live
Seedling 9	2005.009	Dead
Seedling 10	2005.010	Live
Flatlands Clone	2004.001	Live
Nursery Cutting	2004.003	Live
Tedside Original	2004.002	Dead

Two primer pairs produced a total of 121 scorable DNA markers of which 114 were polymorphic (representing 94.23% of all bands) (Table 5.24).

Table 5.24 Total population in 2004, the number of bands and degree of polymorphism

Primer pairs + fluorescent marker	Total bands	Polymorphic bands	% Polymorphism
Mse I **CAT** / EcoRI **AGG**	61	56	91.8%
Mse I **CTC** / Eco RI **AAC**	60	58	96.66%
Total	**121**	**114**	**94.23%**

The degree of polymorphism in the population was reduced when the original plant and seedlings 4, 5, & 9 were destroyed in a landslide on the island in January 2005 (Table 5.25).

Table 5.25 Total population in 2005, the number of bands and degree of polymorphism

Primer pairs + fluorescent marker	Total bands	Polymorphic bands	% Polymorphism
Mse I **CAT** / EcoRI **AGG**	59	50	84.74%
Mse I **CTC** / Eco RI **AAC**	54	50	92.60%
Total	**113**	**100**	**88.60%**

Comparisons between the extant population in 2006 and the original population in 2004 show that the number of polymorphic bands was reduced by 14 (from 114 marker bands to 100 marker bands) and the percentage of polymorphism lost from the population was 5.6% (Table 5.26).

Table 5.26 Percentage polymorphism loss in *Abutilon pitcairnense* population in one year due to environment stochasticity

Primer pairs + fluorescent marker	% Polymorphism 2004	% Polymorphism 2006	% Loss of polymorphism (between 2004 -2006)
Mse I **CAT** / EcoRI **AGG**	91.8	84.7	7.0
Mse I **CTC** / Eco RI **AAC**	96.6	92.6	4.0
Total	**94.2**	**88.6**	**5.6**

The contribution of each individual to the overall diversity within the population is outlined in Figure 5.11. The individual that produced the most bands in the analysis was the wild original specimen from Tedside. The cuttings from this original plant which are termed the clone at Flatlands and the nursery cutting have preserved the diversity of this now extinct original wild plant. Of all the seedlings produced Seedling 6 (now planted at Pulau) and Seedling 3 (currently in the nursery) had the next largest number of bands followed by the now extinct seedling 5.

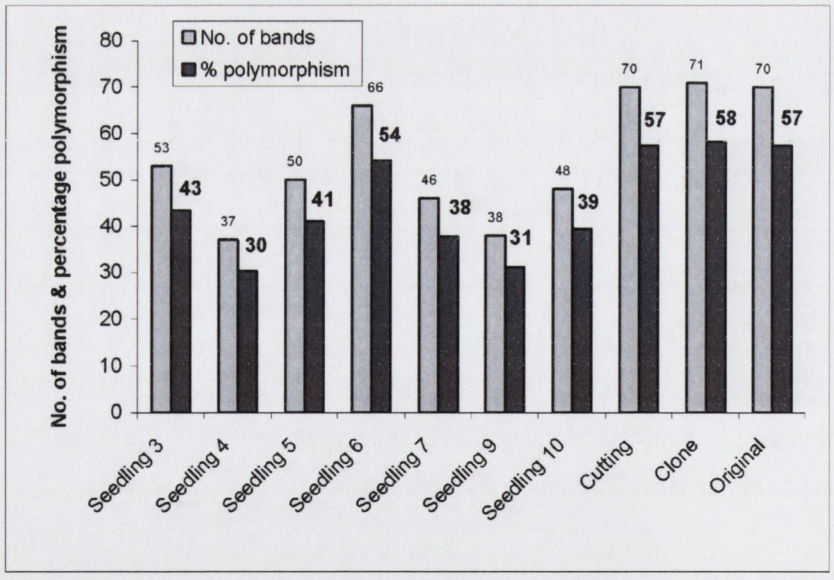


Figure 5.11 No. of loci bands and percentage polymorphism

With the current extant population Shannon's diversity index (0.5006) remains high and Nei's (1973) total observed diversity (0.3465) is also remains high (Table 5.28). No between population statistics

were calculated or F-statistics with only one population on the island representing 100 % of the global population of this species.

Table 5.28 Summary genetic statistics for extant and extinct population of *Abutilon pitcairnense*

		2004	2006	Difference
Sample size	**n**	**10**	**6**	**4**
Shannon diversity Index	***Is***	0.5431	0.5006	0.0425
Total observed diversity	H_T	0.3730	0.3465	0.0265
Within population diversity	H_s	0.3730	0.3465	0.0265

A matrix to demonstrate the genetic distance and genetic identity of individuals in the population was calculated using Nei's (1978) unbiased measures of genetic identity and genetic distance (Table 5.27) The maximum genetic distance was between the seedling 3 and seedling 9 (0.20) outlined in bold.

No genetic distances found were between Tedside original, the Flatlands clone and the nursery cutting showing that propagation by cuttings can provide replicates in order to build up numbers of distinct genotypes. All genetic distances which were greater than 0.10 are also highlighted in bold, the seedlings lost in the landslide seedlings, 4, 5 and 9 were unfortunately some of the most genetically distinct individuals, and these are highlighted in green. However, the remaining, seedlings 3, 6, 7 and 10 are still somewhat genetically distinct from the original genotype.

Table 5.27 Nei 's unbiased measure of genetic distance calculated for *Abutilon pitcairnense* .

	S5	S6	S7	Tedside	Flatlands	Nursery	S3	S4	S9	S10
S5	0									
S6	0.06	0								
S7	0.10	0.06	0							
Tedside	0.11	0.12	0.12	0						
Flatlands	0.11	0.12	0.12	0	0					
Nursery	0.11	0.12	0.12	0	0	0				
S3	0.06	0.04	0.04	0.11	0.11	0.11	0			
S4	0.12	0.09	0.09	0.17	0.17	0.17	0.06	0		
S9	0.15	0.15	0.15	0.16	0.16	0.16	0.20	0.19	0	
S10	0.14	0.10	0.13	0.10	0.12	0.12	0.12	0.13	0.11	0

A summary unweighted pair-group with arithmetic averages (UPGMA) tree was constructed (Figure 5.12) using mean character difference and Nei-Lei (Equation 5.2) (1987) genetic distance (PAUP 4.0), and bootstrap analysis was also used to interpret the genetic relationships between the samples. The resulting tree shows similar results to Table 5.27.

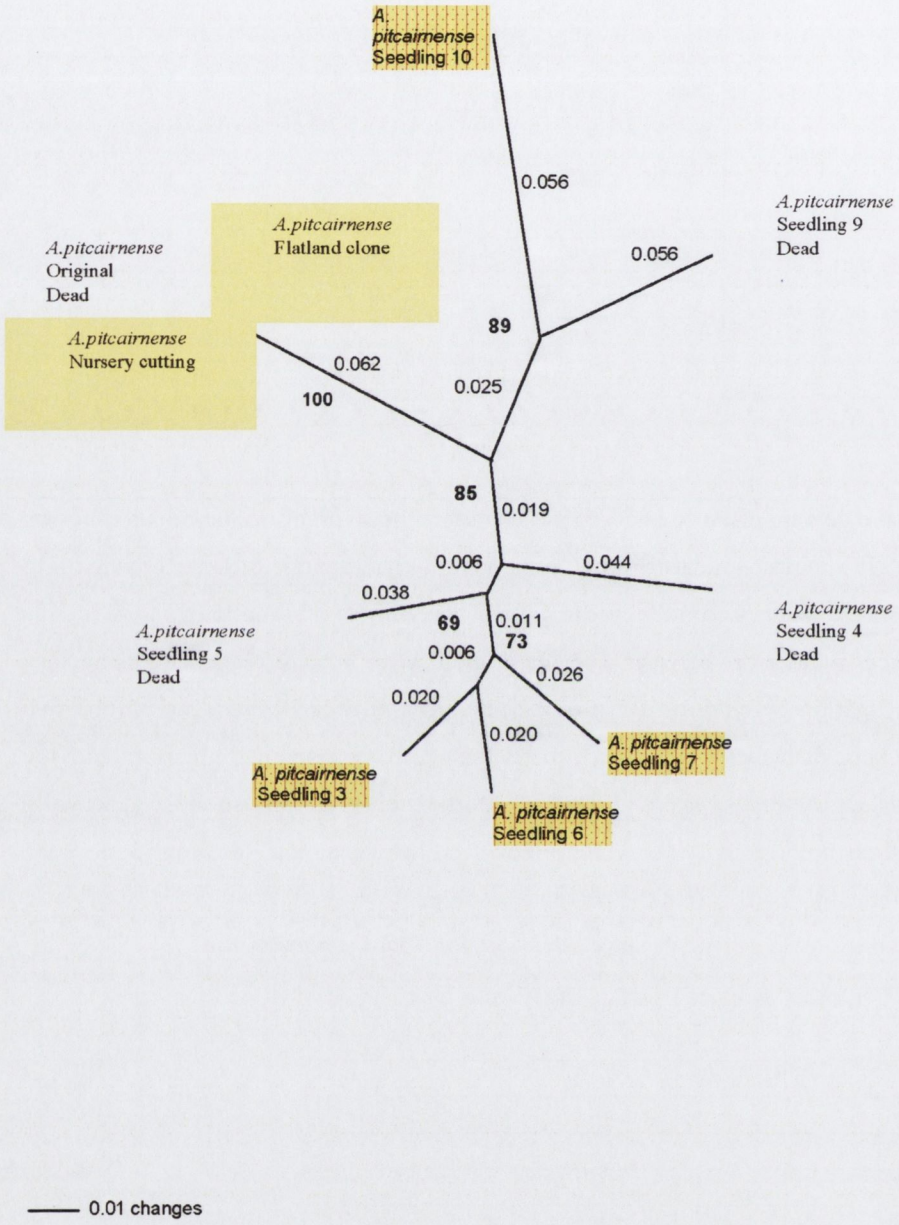


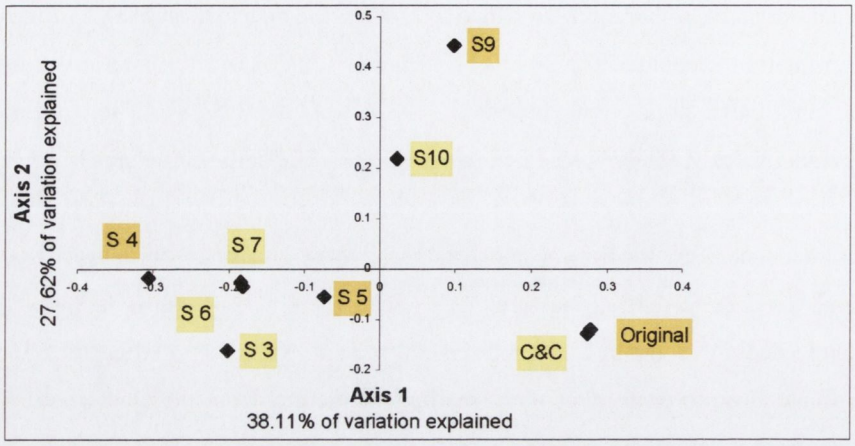
Figure 5.12 UPGMA unrooted dendrogram of *Abutilon pitcairnense* with bootstrap values (highlighted bold). Extant seedlings are highlighted in yellow and extant cuttings (clones of the original) are highlighted in orange.

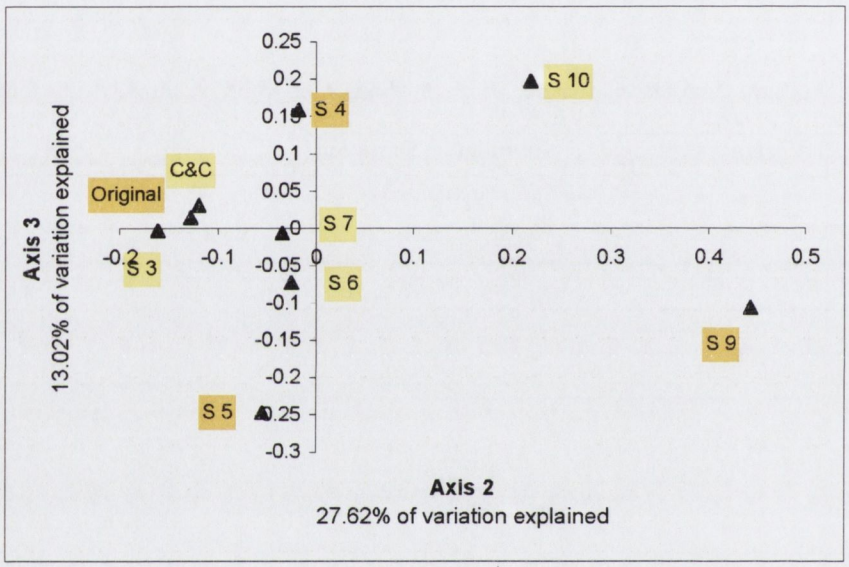
A PCO ordination was carried out using 1- Sørensen Distance to arrange the distribution of individuals. The first three axis of the ordination accounted for 38.1%, 27.6% and 13.02% of the variation in the data respectively with a cumulative total explanation of 78.76% (Table 5.28). The original rediscovered *Abutilon pitcairnense* at Tedside ordinated close to the Flatlands clone and nursery cutting.

Two of the now extinct seedlings, seedling 4 and seedling 9 were shown to be distinct from both the other seedlings and the original and clonal material. Seedling 4 was the most negatively correlated with axis one and seedling 9 the most positively correlated with axis 2 (Figure 5.13). Seedlings 3, 7 and 6 all ordinate close to each other with seedling 10 distinct from the cluster and all seedlings are distinct from the original clonal and cutting plants.

Table 5.28 Eigenvalues, variance and cumulative variance of the ordination of Abutilon pitcairnense data obtained from AFLP analysis

	Eigenvalue	Variance explained %	Cumulative Variation explained %
Axis 1	0.447	38.10	38.11
Axis 2	0.320	27.62	65.74
Axis 3	0.143	13.02	78.86





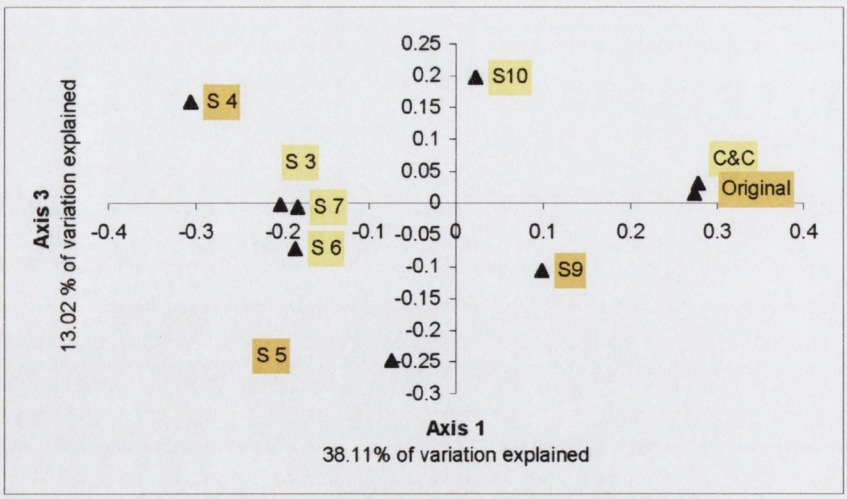


Figure 5.13 PCO ordination of data obtained from clones and seedlings of Abutilon pitcairnense from AFLP analysis. Extant seedlings and cuttings (clones of the original) are highlighted in yellow

5.3.4. *Coprosma benefica*

Population Survey

Coprosma benefica is a critically endangered endemic species on Pitcairn Island. It was selected for detailed study by Kingston (2001). It occurs in one population on the island spread over an area of $500m^2$. The population consisted of 12 individuals in 1997, and a population census in 2005 revealed that the population number had dropped to 11 individuals The species is dioecious and consisted of 7 females, 1 male and 4 of undetermined sex in 1997. One large male (97-655) recorded in 1997 was found dead in 2003. In 2005 the population consisted of 8 females, 1 male and 2 individuals showing male and female expression (Table 5.29)

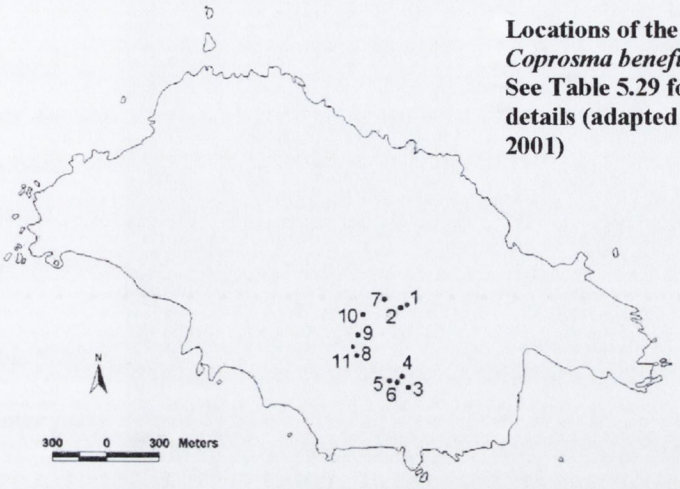
The population is highly threatened due to a road expansion schemes and the constant threat of wind-throw from SW gales. It occurs in *Lantana camara* scrub, fernlands and settlement areas on the island (Kingston 2001). The population information from 1997 is summarised in Kingston (2001) and the most recent survey information (2005) is summarised in Table 5.29. A location map, habit (Plate 12) and profile picture are presented (Plate 13).

Plants of *Coprosma benefica* were propagated inclusive of all the surviving individuals by seed, cuttings and wildlings. They were planted in plots where *Syzygium jambos* was removed (see Chapter 4) and had a high percentage survival in plots (92%). Desiccation was the main cause for its mortality in plots. Positive height and basal diameter growth rates were recorded with a mean height growth of 42.80cm across all treatments. It grew best in the cut and unweeded treatments (Figure 4.12 and 4.13).

A new population was established with 1-4 individuals of each individual genotype in the existing population at a new site at the Big rock-Pulau, such actions are recommended by Page *et al.* (1995). In addition two vegetative propagated clones from each individual were added beside each of the original plants in 2005 to buffer genotypes against environmental stochastic effects.

Table 5.29 Distribution and population details for *Coprosma benefica* (2005)

Census	Genetic code	Sex	GPS	Description
04-011 **Plant 11**	**C11**	♀	S25°04.302 W130°06.138	Mature tree to 4m
04-012 **Plant 10**	**C10**	♀	S 25°04.302 W130°06.138	Mature young tree to 2m
04-013 **Plant 8**	**C8**	♀	S25°04.519 W130°06.163	Multistemmed
04-014 **Plant 9**	**C9**	♀	S25°04.519 W130°06.163	Mature tree
04-016 **Plant 4**	**C4**	♀	S25°04.477 W130°06.294	Mature small
04-015 **Plant 3**	**C3**	♀	S25°04.477 W130°06.294	Mature large tree to 6m
Dead			S25°04.277 W130°06.195	No evidence of resprouting
04-016 **Plant 5**	**C5**	♂	S25°04.320 W130°06.230	Mature tree to 4m
04-019 **Plant 7**	**C7**	♂ (90%) & ♀ (10%)	S25°04.419 W130°06.173	Mature tree to 3m flowers profusely
04-019 **Plant 6**	**C6**	♀	S25°04.427 W130°06.264	Shrub to 5m flowers and fruits profusely.
04-017 **Plant 2**	**C2**	♀ (20%) & ♂ (80%)	S25°04.420 W130°06.283 Taro ground next to *Cerbera manghas* & guy wire	Tree to 4m, old trunks resprouting freely
04-018 **Plant 1**	**C1**	♀	S25°04.419 W130°06.280	Tree to 3m flowers & fruits profusely



Locations of the population of *Coprosma benefica* See Table 5.29 for individual details (adapted from Kingston 2001)

Plate 12 Map locations of census individuals of *Coprosma benefica* (above) and habit in scrub vegetation (marked with red triangle) with *Lantana camara* in the foreground and Citrus *aurantica* to the left.

Plate 13 ***Coprosma benefica*** **female plant in flower and fruit**

Genetic Analysis

Samples for genetic analysis were obtained from the extant population in 2004. DNA was extracted from the leaf material and quantified using techniques outlined in Section 5.2.3 & 5.2.4 and AFLP reactions outlined in Section 5.2.5. The living samples investigated are listed in Table 5.29 (2005 census). Two primer pairs produced a total of 246 DNA markers of which 237 were polymorphic (representing 96.34% of all bands) (Table 5.30).

Table 5.30*Coprosma benefica* population in 2005, the number of bands and degree of polymorphism

Primer pairs + fluorescent marker	**Total bands**	**Polymorphic bands**	**% Polymorphism**
Mse I **CAT** / EcoRI **AGG**	120	112	93.33
Mse I **CTC** / Eco RI **AAC**	122	118	96.72
Total	**242**	**230**	**95.00%**

The contribution of each individual to the overall diversity within the population is outlined in Figure 5.14. The individual with the most loci bands is Plant 8 (Female) (134 bands) in McCoy's Valley under threat from wind throw and falling banana plants in the vicinity. The individual with the least number of loci bands was plant 4 (Female) (40 bands).

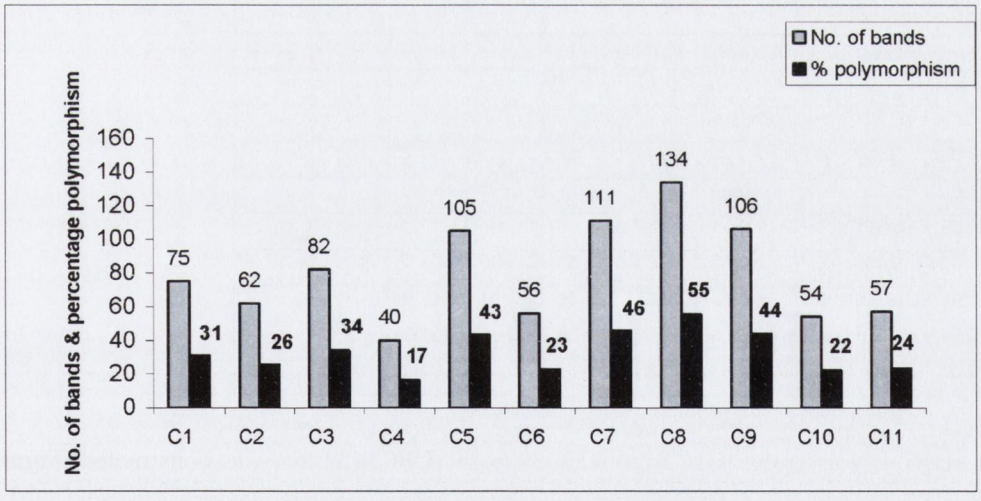


Figure 5.14 Percentage polymorphism and number of loci for individual *Coprosma benefica*

With the current extant population Shannon's diversity index (0.5055) remains high and Nei's (1973) total observed diversity (0.3354) is also remains high (Table 5.31). No between population statistics were calculated or F-statistics with only one population on the island representing 100 % of the global population of this species.

Table 5.31 Summary genetic statistics for extant and extinct members of the population of *Coprosma benefica*

		1997 RAPD Analysis	2005 AFLP Analysis
Sample size	**n**	**12**	**11**
Shannon diversity Index	***Is***	0.168	0.505
Total observed diversity	H_T	0.107	0.335
Within population diversity	H_s	0.107	0.335

A matrix to demonstrate the genetic distance and genetic identity of individuals in the population was calculated using Nei's (1978) unbiased measures of genetic identity and genetic distance (Table 5.32) The maximum genetic distance was between the plant 4 and plant 1 (0.72) underlined in Table 5.32.The minimum genetic distances found were between plants 5, 7, 8 and 9 (<0.50) which are outlined in bold. All genetic distances which were less than 0.50 are also highlighted in bold.

Table 5.32 Genetic distance between the eleven remaining individuals of *Coprosma benefica* in 2004

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
C1	0										
C2	0.53	0									
C3	**0.48**	0.5	0								
C4	0.72	0.65	0.69	0							
C5	0.59	0.63	0.60	0.73	0						
C6	0.51	0.51	0.53	0.62	0.69	0					
C7	0.59	0.59	0.56	0.78	**0.39**	0.7	0				
C8	**0.48**	0.62	**0.49**	0.78	**0.45**	0.63	**0.34**	0			
C9	0.53	**0.46**	0.53	0.70	**0.46**	0.57	**0.37**	**0.41**	0		
C10	0.59	0.58	0.50	0.69	0.66	0.63	0.68	0.61	0.62	0	
C11	**0.44**	0.56	0.48	0.65	0.65	0.51	0.66	0.58	0.59	0.58	0

A summary unweighted pair-group with arithmetic averages (UPGMA) tree was constructed (Figure 5.14) using mean character difference and Nei-Lei (1987) genetic distance in PAUP 4.0, and bootstrap analysis was also performed. The tree further emphasis the close relationships between plants 5, 7, 8 and 9, two of this group show male expression. This group is genetically distinct from the other main cluster of individuals 1, 2, 3, 6, and 11 in this group only one plant has male expression.

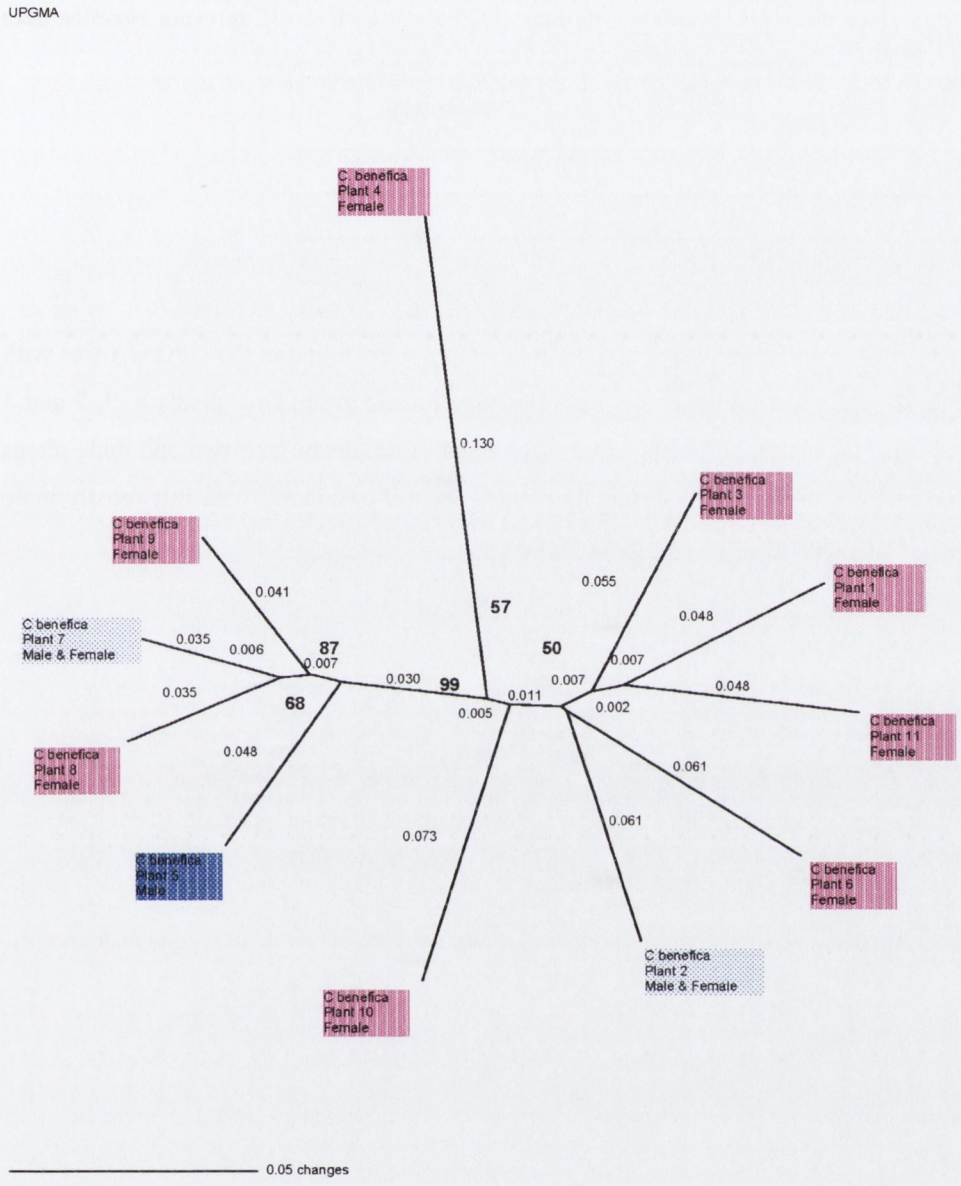


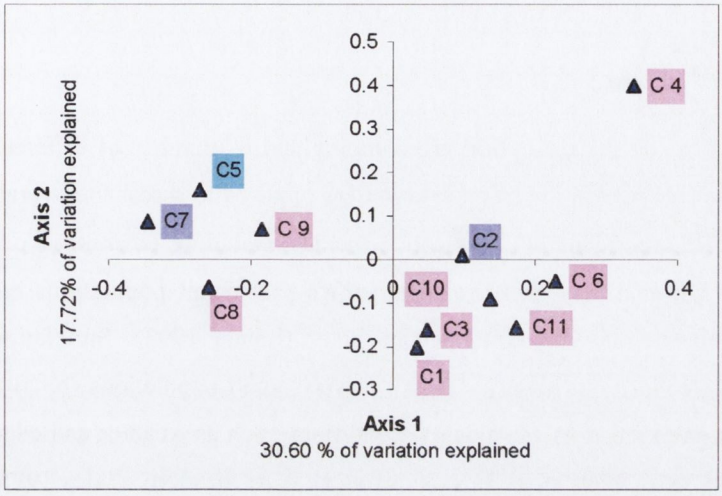
Figure 5.14 UPGMA unrooted dendrogram of *Coprosma benefica* with bootstrap values (highlighted in bold).

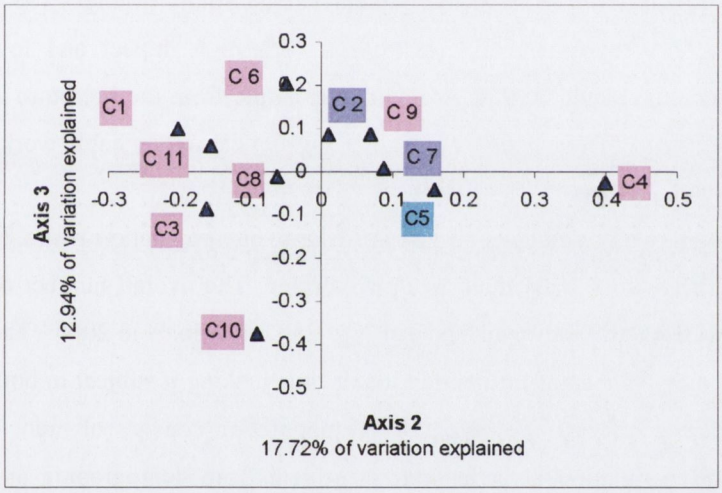
A PCO ordination was carried out using 1- Sørensen Distance to arrange the distribution of individuals. The resulting clustering of individuals with ordination (Figure 5.15) was similar to the clusters outlined in the UPGMA dendrogram using Nei-Li genetic distance measure (Figure 5.14). The first three axes of the ordination accounted for 61.28% of the cumulative variation in the dataset examined. Axis 1 accounted for 30.60%; Axis 2 for 17.72% and Axis 3 12.94%.

Table 5.33 Eigenvalues, variance and cumulative variance of the ordination of Coprosma benefica data obtained from AFLP analysis

	Eigenvalue	Variance explained %	Cumulative Variation explained %
Axis 1	0.523	30.60	30.60
Axis 2	0.303	17.72	48.33
Axis 3	0.221	12.94	61.28

Axis one and axis two of the ordination separated the two main groups found in the UPGMA tree with plants 3, 1, 11, 6, 2, 10 and 4 having positive values on axis one and group two, plants 8, 9, 7 and 5 with negative values on axis 1. Female plants had mostly negative values on axis two and male plants had mostly positive values on axis two with female plants, 4 and 9 ordinating with this mostly male group. Axis 3 again clearly separated the two main groups.





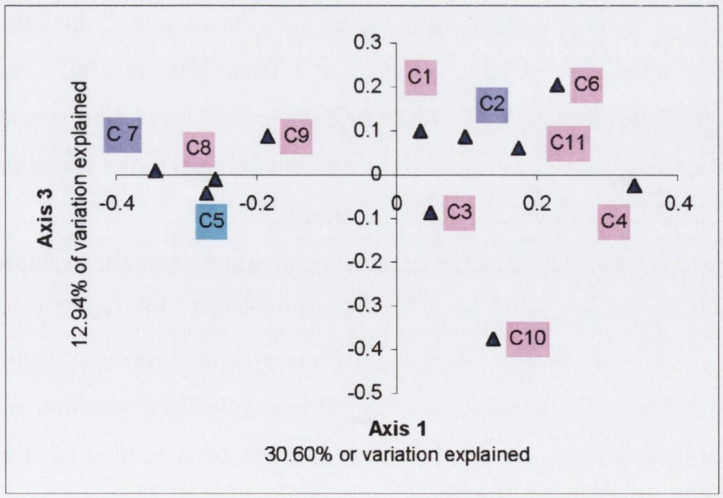


Figure 5.15 PCO ordination of data obtained from clones and seedlings of *Coprosma benefica* from AFLP analysis. Male plants are highlighted in blue and plants showing both male and female expression are highlighted in purple.

5.4 Discussion

5.4.1 *Lastreopsis* c.f. *pacifica*

The range of processes that may lead to species extinction are complex and a number of different indicators such as rarity, rate of decline and population fragmentation are required to assess the overall risk (Hartley & Kunin 2003). On Pitcairn Island, many of the native species, including *Lastreopsis* c.f. *pacifica* exist only in a few sites in low numbers. Research has shown that small populations are generally more prone to demographic and environmental stochasticity (Lande 1993). Simulation studies have shown that demographic stochasticity is only relevant in very small populations (N<50). Environment stochasticity depends on variation in environmental conditions such as weather and edge effect as habitat becomes more fragmented, which magnifies environmental stochasticity (Jules 1998; Lande 1998). Populations need to be large to buffer against environmental stochasticity (Menges 1991; Menges 1992; Lande 1993; Lande 1998) environmental catastrophes with high impact and low frequency may easily cause local extinction (Lande, 1993). A dramatic example from the literature is the extinction of the roadside populations of *Phyteuma spicatum* subsp. *nigrum* caused by cable works in road verges (Oostermeijer *et al.* 1998).

Lastreopsis c.f. *pacifica* exists in two populations on Pitcairn Island, one population with 276 individuals at Browns Water and another with 9 individuals at Pulau Bridge. The overall number of individuals in the population has grown from 189 individuals in 1997, to 285 individuals in 2005. The Brown's Water population consists of only 37 mature fertile individuals thus making it subject to both demographic and environmental stochastic effects. The Pulau Bridge population consists of mere 4 fertile mature individuals. This small population is under extreme threat from demographic and environmental stochastic effects. The Pulau Bridge population however, has shown some population expansion where the population growth rate varied from $\lambda = 8$ to $\lambda = 1$ from 1997 to 2005. This population was not recorded during fieldwork in 1991 (S. Waldren *pers. comm.*) and this species appears to be expanding its range on Pitcairn Island and appears to be a recent colonist to the island the species is found naturally occurring in Samoa, the Cook and Society Islands.

Dramatic changes in growth rate have also been noted in other small populations for example, a study on *Ipomopsis aggregata* populations had growth rates vary from 0.55 to 12.88 over 2 years (Heschel & Paige 1995). The larger population at Brown's Water growth rate varied less significantly from λ=1.388 to λ=1.018 from 1997 to 2005. The population at this site may well be reaching full carrying capacity. λ values around 1 are considered "stable". Conversely, if λ<1 for a number of years in a row the population may be at risk (Oostermeijer *et al.* 2003).

The decrease in λ to 1.018 in the Browns Water population, from 2004 to 2005, while this trend is not alarming the population should be monitored every few years to ensure the population remains stable and to ascertain the range expansion of this species on island. The overall score for the population which includes information on the quality, condition, long term prospects and site protection against extrinsic human factors is marginal to good.

The Brown's Water population is nestled on volcanic tuff cliffs at the bottom of one of the main island tracks to Flatlands (Garden area) and Tedside (a popular location for fishing). The rock bank is bound with roots of native *Homalium taypau* and increasingly invading *Syzygium jambos* roots. *Syzygium jambos* has shallow roots on Pitcairn Island which causes soil between the roots to harden in dry weather and prone to washout during heavy downpours. Road expansion works have also loosened the stone and soil at the top of the cliff face and have increased the erosion potential at the site. Care should be taken with any future road works not to loosen the bank any further. The Pulau Bridge population is under threat from heavy rains and stream bursts as the individuals grow on the moist streamside bank. The area itself is little visited by the islanders and is >10m from the road verge. Heavy shade provided by the *Syzygium jambos* forest (canopy cover (>70%) has not adversely influenced the number of individuals in the population over the years 2003 to 2005. This location should be monitored for any damage to the population after heavy rains.

5.4.2 *Haloragis* sp.

Haloragis sp. was first recorded as occurring on Pitcairn Island in 1997. The population reported at Tautama in 1997 appears to be expanding into nearby southern cliffs. The effective population size at the three populations investigated is well above recommendations for a minimum viable population (N_e>50) and free from the threat of demographic stochasticity (Lande 1998). *Haloragis* sp. was also found colonizing areas which were recently exposed due to erosion. This plant is most likely a relatively recent colonist on the island and is capable of regenerating and colonizing recently eroded areas. The main population however, is situated on a very steep bank and if a large scale land slip occurred at this site the population numbers would be severely reduced.

It grows in open coastal shrub and cliffs but was not found in very dense swathes of grass, though only in areas where rank vegetation had not yet become dominant. It would be interesting to observe the fate of these populations on recently eroded areas to judge whether increased competition from grasses would cause a decrease in its population size. The current score for the three populations investigated which includes information on the quality, condition, viability and defensibility of the population and sites is good.

5.4.3 *Myrsine* aff. *niauensis*

In conservation biology the desire is to seek and maintain all distinguishable taxa whether or not there is full agreement on the definition of a species (Hunter 1996). In this study the morphological character and genetic character of *Myrsine aff. niauensis* was investigated in order to clarify its specific status. This sole individual on Pitcairn Island is reproductively isolated from the next nearest "species" on Henderson Island. *M.* aff. *niauensis* was found to differ from *M. hosakae* genetically with the proportion of nucleotides that differed between the two species 0.0072. Specimens from other Pacific island *Myrsine* were obtained from the Bishop Museum in Hawaii for comparison with the Pitcairn *Myrsine*. The only common feature to all of the *Myrsine* specimens examined was the leaf and petiole. Measurements were taken of the leaf and petiole and *Myrsine* aff. *niauensis* was found to differ from the other specimens examined (Figure 5.9) with larger leaves and petioles, further more detailed measurements and analysis are required to elucidate if these differences in leaf length and width and petiole length and width are of relevance as leaves and petiole sizes can be extremely variable even within species. While the results presented of leaf and petiole measurements and sequence data do indicate towards *Myrsine* aff. *niauensis* being distinct, the results cannot be taken as conclusive. However, every effort must be made in conservation terms to maintain this sole taxon on Pitcairn Island and protect it from environmental stochasticity. The plant itself is currently showing male expression and with no female plants found on the island in 2003, 2004 or 2005 it is a species which can be considered one of the "living dead".

The habitat this sole individual resides in is extremely threatened by environment stochasticity. Its score ranking is poor, as it sits on top a crumbling and eroding clay bank and road maintenance works in 2005 almost caused its demise. The plant was marked with luminous twine and labelled in 2005 and every islander was made aware of its location. Cuttings were taken and *ex-situ* collections of this genotype are now conserved in Pitcairn Island nursery, Trinity College Botanic Gardens and at the National Botanic Gardens, Glasnevin, Dublin.

5.4.4 *Abutilon pitcairnense*

The original rediscovered *Abutilon pitcairnense* is currently thought to be extinct in the wild since 2005. However, the genotype was conserved through propagation (Figure 5.13). Seed set in *Abutilon pitcairnense* is infrequent, out of the two flowers on the Flatlands clone in 2003, one seed capsule developed which contained 50 seed of which 10 germinated and 3 of these seedlings died soon after germination. Island plants are sometimes known to have low germination rates (Frankham 1998).

Annual hand-pollination of the clone in cultivation at Trinity College Dublin has failed to result in seed set. A planting scheme where three of the seven seedlings (4, 5 & 9) were planted in the

vicinity of the sole wild individual also failed when a landslip wiped out the entire group in also January 2005. Planting out seedlings 4, 5, and 9 beside the original plant found in 2003 in hindsight was not wise. This was an attempt to increase the geneotypic diversity at the original site for future progeny. This was also done to help increase the chances of sexual reproduction as the lack of sexual reproduction in the natural populations can often be as a result of limited genotype diversity (Godt & Hamrick 1999). The percentage loss of polymorphism (5.6%) due to the deaths of four of the eight genetically distinct individuals was not as dramatic as it could have been considering the genotypic population size was decreased by 50%. The clonal individuals, the flatlands clone and nursery clone analysed, retained many of the polymorphic bands of the original wild plant. Despite the critically low numbers of clonal individuals and seedlings remaining of *Abutilon pitcairnense*, the percentage polymorphism remains high at 88.60 %. When this value is compared percentage polymorphism values obtained from the literature for other rare and endangered species, for example, *Limonium dufourii*-20.2% (Palacios *et al.* 1999) *Wollemia nobilis*-0% (Peakall *et al.* 2003), *Scalesia affinis*-54.8% (Nielsen 2004) *Meterosideros bartletii*-65.4% (Drummond *et al.* 2000) it still remains high or equivalent, for example, *Leucopogon obtectus* ~ 90% (10 individuals) (Zawko *et al.* 2001).

A. pitcairnense has been successfully propagated by both seed and cuttings, and continued attempts should be made to maintain the current number and obtain seed from individuals. A breeding programme to cross the most genetically distant individuals (Table 5.27) should be performed. Results from the genetic analysis performed reveal that crossing seedling 3 with the Flatland clone, and seedling 10 with seedling 6 (Table 5.27) would help maximise the genetic diversity, as dissimilar pairs may produce fitter than average offspring (Amos & Balmford 2001).

The remaining individuals with the highest number of polymorphic loci (Flatlands clone and Seedling 6) should be maintained in both *in-situ* and *ex-situ* collections as a priority. Ideally four duplicates of each individual should be maintained to ensure the total genetic variation is accounted for (Page *et al.* 1995) High genetic diversity in rare plants has previously been attributed to a number of factors including insufficient length of time for genetic diversity to be reduced following a natural reduction in population size and isolation (Coates, 1998). It has also been suggested that small populations subject to stressful conditions may loose heterozygosity more slowly than those in benign environments, this is thought to occur when populations are historically small or selfing, as they have purged recessive alleles through inbreeding (Lesica & Allendoft 1992).

5.4.5 *Coprosma benefica*

Coprosma species are found across the Pacific to South America with centres of diversity in New Zealand and Hawaii (Heads 1996). They are commonly dioecious with fruits that are bird dispersed (Lee *et al.* 1994). The dioecious population of *Coprosma benefica* on Pitcairn Island remains critically endangered and under threat from both environmental and demographic stochasticity. The population number on Pitcairn Island has decreased by one during the survey years. The remaining individuals are under constant environmental stochastic threat from south westerly gales which frequently occur during June-September every year. The sex of each individual in the population was determined and the number of male plants recorded from the surveys in 1997 and 2005 shows an increase in the number of male plants from one male in 1997 to three males in 2005 though it remains unclear whether the individuals surveyed in 1997 were the same individuals surveyed in 2005.

AFLP analysis in 2005 revealed 95.00% polymorphism within the species, the previous survey with RAPD in 1997 obtained a value of 35.75% polymorphism (Kingston 2001). More variation was found using AFLP. This phenomenon has been noted in other studies for example, *Limonium dufourii* (Palacios *et. al.* 1999) and *Lens* (Sharma *et al.* 1996) where both the number of polymorphic loci and the heterogeneity per locus found were usually lower for RAPD analysis than for AFLP analysis (Palacios *et al.* 1999). AFLP markers unlike other fingerprinting methods such as RAPD (Parker *et al.* 1998) has high reproducibility (Vos *et al.* 1995) and Nei–Lei approaches to genetic distance measures are also better satisfied with AFLP rather than RAPD (Palacios *et al.* 1999). Shannon's diversity index (I_s) and the total observed diversity H_T was also higher for the population of *Coprosma benefica* on Pitcairn Island using AFLP (Table 5.31). The results obtained from AFLP analysis are also useful to guide a future breeding programme to maximise the genetic diversity of the species. The individuals assessed grouped into two assemblages. The male plant in group one C2 (associated with females C1, C3, C6, C10 and C11) could be crossed with females from group 2; C9 and C8 and visa versa. Males from both groups should be crossed with C4 who emerged from the analysis as a very distinct individual.

Protection for all the existing genotypes found in 2005 was afforded by planting a new population with 1-4 individuals of each genotype at a *in-situ* conservation site on Pitcairn Island at Big rock-Pulau, such proactive conservation measures were recommended by Page *et al.* (1995). In addition efforts to reduce the threat of environmental stochasticity for this species were made by planting two vegetative propagated clones from each individual alongside existing genotypes.

It is often argued that rare species suffer double jeopardy because they have low population sizes and restricted ranges (Lawton 1993; Johnson 1998). To improve risk assessments and provide sensible advice for conservation planning we have to go beyond these general statements and develop a better understanding of the individual species and there ecology. The six species investigated in this

chapter demonstrate that to move beyond the "crisis" of what to do with critically small populations monitoring species over a period of time can reveal different perspectives. For example, the populations of *Lastreopsis* c.f. *pacifica* and *Haloragis* sp. are most likely to be recent colonists to Pitcairn Island and their range appears to be expanding and thus conservation efforts for these species can be based solely on monitoring.

The other three species investigated; *Coprosma benefica*, *Myrsine* aff. *niauensis* and *Abutilon pitcairnense* require a more hands-on approach to build up the number to reduce their susceptibility to the constant stochastic threats of wind-throw and landslide. Molecular analysis has revealed distinct genotypes within *Coprosma benefica* and *Abutilon pitcairnense*, and these data can be used to inform a breeding programme for each species to maximise genetic diversity alongside building up numbers. The problems of conclusively identifying to species level plants found in remote locations was highlighted with investigations into the taxonomy of the sole individual of *Myrsine* aff. *niauensis* on Pitcairn island. Leaf morphology alone was not sufficient to conclusively identify which *Myrsine* species it is. Sequencing analysis (*trnL-f*) revealed only its close relationship to *Myrsine hosakae* on Henderson Island, whether or not it is a distinct endemic species to Pitcairn Island still remains unanswered.

5.5 General conclusions

When species exist in critically low population numbers the greatest threats are considered stochastic in nature. The species investigated in this chapter differ in both their conservation requirements and degree of threat into the future and no general conservation statement can be made on small populations without through investigations and monitoring of population demographics and genetic diversity. Two of the species investigated *Lastreopsis* c.f *pacifica* and *Haloragis* sp. were found to be increasing in number, and it is thought that these populations are recent colonists to the island and may in fact be increasing their populations on the island. The steadily increasing population numbers abate conservation concern for these species. The species refound during this project *Abutilon pitcairnense* and *Myrsine* aff. *niauensis* are however of great conservation concern, and they are under the constant threat of environmental stochastic events, which was highlighted dramatically during the project when a landslide destroyed the sole surviving wild individual of *Abutilon pitcairnense*. Island species however, are remarkably more resilient than often credited, with the genetic diversity in both *Abutilon pitcairnense* and *Coprosma benefica* high in comparison to other critically endangered continental populations, such as the infamous *Wollemia nobilis* which demonstrated no genetic diversity in studies and *Limonium durfourii* which showed only small levels of genetic diversity. The importance of using improved molecular techniques was highlighted with *Coprosma benefica*. A previous investigation using RAPD revealed only 35.75% polymorphism within the population, using an updated molecular technique AFLP, the percentage polymorphism within the population was found to be 95%.

Chapter 6

Discussion and future recommendations

6.1 Introduction

The main aims of this thesis were to quantify the effects of the invasive species *Syzygium jambos* on the biodiversity of Pitcairn Island and to investigate and develop a suitable method for control, and initiate a forest recovery programme after *Syzygium jambos* control. These aims were met in Chapters 2, 3 and 4 with the main findings from each chapter summarized briefly below.

Chapter 2 outlined how the soils and vegetation were affected by the presence of *Syzygium jambos*. These results indicated that both the species diversity and soil chemistry were negatively affected by the presence of *Syzygium jambos*.

In Chapter 3 pilot experiments were carried out using two different physical control methods and two different chemicals on *Syzygium jambos* and assessing the mortality with each treatment. Both the physical and chemical methods used proved successful in controlling *Syzygium jambos*.

Investigations into the make-up of the soil seed bank in the trial plots revealed the lack of native plant species in the soil seed bank of invaded *Syzygium jambos* forest. This indicated that a secondary invasion by weedy species was the likely replacement vegetation after *Syzygium jambos* control. This indeed proved to be the case with very few native and naturalised species found regenerating in the trial plots.

Chapter 4 dealt with forest recovery, with a suite of native and naturalised species propagated and planted into plots where *Syzygium jambos* was controlled. All the species planted demonstrated positive growth rates indicating both the possibility and suitability of native and naturalised species for forest recovery efforts on the island.

Secondary aims were to ensure the populations of endemic and extremely rare plants on the island, as highlighted by Kingston (2001), were conserved with investigations into their population demographics and, when extremely rare, characterization of individual genotypes. These aims were met in Chapter 5 where ongoing investigation into the population demography's and continued threats, which face five of the most critically endangered species on the island were conducted.

Populations of *Lastreopsis* c.f *pacifica* were found to be stable and increasing and appeared in sufficient numbers to negate any immediate effects of demographic stochasticity, with environmental stochasticity discovered to be the main threat to one of the population strongholds at Brown's Water.

This species is considered to be a recent colonist on the island (Kingston 2001, Waldren *pers. comm.* 2007).

The population census of the unidentified *Haloragis sp.* which was a new record for the island in 1997 revealed that this population was also expanding along the southern cliffs. The population numbers in this case were also found to be sufficient to ward off any demographic stochastic effects. The threat of environmental stochasticity to the population was also considered small as they were found colonising in areas which had previously been eroded.

Myrsine aff. *niauensis* which was rediscovered during the project is also presumed endemic and was successfully propagated by cuttings. Three ex-situ collections of this genotype now exist, which help to somewhat negate the large threat of environmental stochasticity. Investigations into its morphology and genetic identity did not however, conclusively prove a distinct identity for this Pitcairn Island plant.

The endemic *Abutilon pitcairnense* was also successfully propagated and two ex-situ collections now exist. The usefulness of horticultural techniques in. preserving genotypes was highlighted when the original and only existing wild plant of this species on the island in 2003 was lost in a landslide in early 2005. Seed was also obtained and grown and AFLP analysis revealed that five distinct genotypes of this species are now successfully conserved in cultivation.

The population of the endemic *Coprosma benefica* was found to have decreased from 12 individuals in 1997 to 11 individuals in 2005. The genotypes which remained in 2005 were characterised by AFLP analysis and a new population which includes all the existing genotypes was planted in 2005 at Big Rock Pulau *in-situ* conservation site. A number of different genotypes were also planted in plots after *Syzygium jambos* was controlled and they showed both high survival and positive growth. The immediate threat of demographic stochasticity has now been abated for this species.

6.2 Recommendations for future *Syzygium jambos* control on Pitcairn Island

The three criteria Myers *et al.* (2000) highlighted as necessary for a successful species control programme were that the species in question is easy to find, easy to kill , and has a non-persistent seedbank. *Syzygium jambos* proved easy to find, relatively easy to kill, though it was present in the seedbank. Based on the results of this study and a review of the finding of other studies some recommendations are made for future control efforts of *Syzygium jambos* on Pitcairn Island.

Initial effort to control Syzygium jambos on Pitcairn Island should be in invaded forest patches closest to native forest community remnants.

1. The native communities in the vicinity of *Syzygium jambos* are most severely threatened given the highly productive and competitive nature of *Syzygium jambos* as mean densities recorded for seedlings ($47.7m^{-2}$), saplings ($6.1m^{-2}$) and adults ($2.3m^{-2}$) are extremely high.
2. Native species diversity in invaded *Syzygium jambos* forest stands closest to native forest communities is significantly higher ($P<0.001$) than native species diversity in *Syzygium jambos* dominated stands further away from native forest communities. Thus control efforts in the vicinity of native forest stands would aid the conservation of many remaining native species.
3. Natural native and naturalised species regenerating were only found *Syzygium jambos* controlled plots which had native species or naturalised species in the vicinity. These results were similar to findings by Odgen *et al.* (2005).

The most suitable physical control method for Syzygium jambos in the future should be frilling and the most suitable chemical control method should be high volume applications of Round-up.

1. Mortality in 2005 was highest (98.8%) for *Syzygium jambos* which were frilled and chemically controlled with Round-up.
2. Secondary weed invasion in frilled plots (18%) was significantly ($P<0.001$) less than in cut plots (71.1%).
3. In order to re-establish a native ground flora frilling appeared to aid the regeneration of native ferns which were found to more frequently in frilled plots (Figure 3.7). The native shrubs *Xylosma suaveolens* and *Cyclophyllum barbartum* were also found to regenerate more freely in frilled plots.
4. The frill treatment requires a smaller number of person hours (66 person hours) than the cut treatment (106 person hours) and is less dangerous for personnel working on steep slopes.
5. The costs for treating *Syzygium jambos* are lower (€523.8) than the start up costs for cut treatment (€767.6).
6. Though the cost of treating with Round-up is marginally more expensive than treating with Tordon. Round-up is considered less damaging to the environment than Tordon, which requires a special permit for use in Hawaii (Motooka *et al.* 2002).
7. Delayed resprouting was a feature of *Syzygium jambos* control efforts. Resprouting was evident with both chemicals used, with Tordon outperforming Round-up in effectiveness. However, the overriding environmental consideration makes Round-up the more favourable chemical and delayed re-sprouting is a feature often noted with invasive species control (Hartman & McCarthy 2004; Dixon *et al.* 2002; Flint & Rehkemper 2002).

8. Forest recovery efforts were more successful in the frill treatment with higher plant survival rates (Table 4.2) recorded for plants in the frill treatment. Individual species also fared better in the frill treatment (Figure 4.8 and 4.9).
9. Mean plant height growth was lower in the frill treatment when compared to the cut treatment, though the differences in height growth rates were not significant between the two treatments when plot weeding was carried out (Figure 4.10).

Time and resource allocation for follow up treatment for resprouting Syzygium jambos individuals and secondary weed species should be incorporated into future control efforts.

1. Twenty-five percent of treated *Syzygium jambos* adults were found resprouting after control efforts. This is a significant figure and future control effort must make allowances for additional treatment for resprouting individuals (Figure 3.3).
2. If the cut treatment practice is used for control of *Syzygium jambos* in the future, efforts must also be made to control the secondary weed invasion which will follow, with weed cover estimates for cut treated plots > 70% after *Syzygium jambos* control (Figure 3.5). There is no point in removing one invasive species to have it replaced with worse (Cronk & Fuller 2001)

6.3 Recommendation for future forest recovery efforts on Pitcairn Island

In forest restoration the aim in many cases is to restore as much of the forest ecosystem structure as possible and re-establishing all the species that live in an original forest ecosystem cannot be replaced in a single step (Elliott *et al.* 2006). This can be considered the case with the Pitcairn Island forest recovery programme initiated in this project. Based on the results of this study the following recommendations are made to aid future forest recovery efforts on the island.

Efforts should be made to control the goat population on the island before further forest recovery efforts are carried out.

Three experimental sites (Site 4 – Tedside to the Sea; Site 5- Lower Mema and Site 6 – Upper Mema) with 210 plants lost due to goat browsing. A further 37% of plant mortality was also attributed to goat browsing (Figure 4.4).

Increased intensive areas for fruit production should be planned for where suitable sites are found in treated Syzygium jambos forest.

1. Historical *Citrus* species and varieties (*C. reticulata, C. aurantifolia, C. medica*) planted in plots had 100% survival.
2. One site at Trevor's Canyon which was planted solely with 234 plants of local *Citrus* varieties also demonstrated 100% survival.

Direct seeding of forest trees is not recommended as a method to aid forest recovery on Pitcairn Island

1. Zero rates of germination success and survival were obtained with direct sowing of two native forest trees *Homalium taypau* and *Meterosideros collina.*
2. Very limited direct sowing success was obtained with *Hibiscus tiliaceus* (10 %)

Direct seeding is often a method which is championed (Cabin *et al.* 2002; Gilbert & Anderson 1998; Guerrant 1996) though more recent studies suggest that it is not suitable for forest recovery effort (Camargo *et al.* 2002; Matthes *et al.* 2003), as was found on Pitcairn.

Native and naturalised species which exhibited rapid growth rates should be used in all further forest recovery effort to suppress secondary invasion by weedy species after Syzygium jambos control.

1. The species recommended for ground cover and weed suppression after *Syzygium jambos* control are: *Hibiscus australense* and *Jasminum didymum* (Figure 4.11).
2. The tree species *Cerbera manghas, Hibiscus tiliaceus, Pandanus tectorius* and *Glochidion pitcairnense* should be used to aid formation of rapid canopy closure which would reduce erosion threats (Figure 4.11).
3. The endemic shrub *Coprosma benefica* and native shrub *Xylosma suaveolens* should be widely planted as they are both infinitely suitable for forest recovery effort and widespread planting would decrease the environmental stochastic threats to remaining genotypes (Figure 4.11).

Further research is required to increase the propagation success of the two native tree species Homalium taypau and Meterosideros collina.

1. No propagation success was obtained with both seed and cutting of *Meterosideros collina* in 2003. Seedlings germinated and survived in 2004. The majority of plants used of *Meterosideros collina* for plot planting in 2004 were wildlings collected around the Island in 2003.
2. Only one island tree of *Homalium taypau* set seed during the project duration. The seed set however, germinated well in the nursery and the resultant seedlings also grew very well. No successes were obtained from *Homalium taypau* cuttings.

The plots used in this research should be revisited and resurveyed at five to ten year intervals.

1. A survey is required to access the feasibility of forest recovery and to learn more about the success of the methods employed. For example, frilled plots may initially outperform cut plots in species survival and weed suppression but this situation could change in time with higher growth rates exhibited for plants grown in cut plots.
2. Soil processes generally take longer to change and with the newly introduced vegetation in the plots it would be interesting to see if soil pH and organic matter increases or decreases under the new cover.
3. It would be important to see if the native ground flora of fern species actually arrives into treated plots or whether further restoration efforts require all three woodland layers, tree, shrub and herb layers to be replaced.

Unfortunately the main source of funding for the current *Syzygium jambos* control project ended in December 2006. The UK government needs provide long term and ongoing support for control and restoration projects in overseas territories. Invasive species control, if it's to be effective at all, requires long term commitments to monitoring to ensure that areas where *Syzygium jambos* was controlled remain that way. Without ongoing support the effort and work to date may prove futile, with *Syzygium jambos* reclaiming much of its former territory and making its control on Pitcairn Island an impossible *sisyphsian* task.

6.4 Recommendations for future species recovery and monitoring

Conservation measures were carried out for the five newly recorded and critically endangered plant species investigated in Chapter 6. While each species investigated required individual actions suited to that species, the main aim was ensure as many of the genotypes or representatives of populations are protected from both demographic and environmental stochasticity.

The next steps for the conservation of the critically endangered endemics *Coprosma benefica* and *Abutilon pitcairnense* are to cross-pollinate the most genetically distinct individuals to maximise the future genetic diversity of these populations. *In-situ* conservation measures are considered the ideal method for species recovery and four of the five the species investigated are now conserved *in-situ* and with some in *ex-situ* collections in botanic gardens.

The nursery facility which was built in 2003 and the plant conservation team Mr. Jay Warren (conservation officer), and Mrs. Carol Warren (caretaker of the Big Rock-Pulau *in-situ* conservation

site), will aid the conservation of critically endangered plant species into the future. The Pulau *in-situ* conservation site has also provided a safe location for many of the islands critically endangered plants and provided an education resource to visitors and provides some employment. It is hoped that any revenues received from visitors to the site will be reinvested in Pitcairn Island plant conservation, and that plant conservation may become a self-sustaining enterprise for the island.

Monitoring of the critically endangered endemic and newly discovered species should be carried out routinely every five to ten years by scientific staff so that an accurate assessment of population fluctuations and ongoing environmental threats can be regularly monitored, and updated plans for continued conservation of these species are drawn up.

6.5 Concluding remarks

Pitcairn Island had a well-studied flora at the end of Kingston's (2001) research and many of the recommendations outlined have been followed through with this project, specifically on the control of invasive *Syzygium jambos*. At the end of this study, Pitcairn Island now has a detailed plan of action for dealing with invasive *Syzygium jambos* and dealing with the consequences of its control. Native and naturalised species on Pitcairn Island can also be successfully established on sites formerly dominated by *Syzygium jambos* indicating the future potential for forest restoration on Pitcairn Island.

The invasive species *Syzygium jambos* is pollinated by introduced exotic pollinator (*Apis mellifera*) and distributed throughout the island by introduced rats. This synergistic invasion has resulted in *S. jambos* successfully spreading to cover over 40% of the land area of the island since its introduction in the late 1800's. In advance of the invasion tide is the remnant native vegetation, many of its species have lost there natural dispersal ability with the demise of the fruit dove, for example *Coprosma benefica* and *Xylosma suaveolens,* these now exist in critically low population numbers. The issue of the other invasive plant and animal species on the island such as goats and rats will have to be addressed at some stage in the future if conservation efforts on the island are to progress. Areas cleared and treated during this project and replanted with native species were also severely grazed by goats.

The severe grazing damage to stands of natural vegetation on the island by the goat population is also of great concern. The remnants of much of the natural vegetation, the source of material for all future restoration work exists along Pitcairn's coastal fringe. The coastal fringe is unfortunately also the main preserve of the goat population. Much grazing damage was noted on the dominant coastal tree, *Pandanus tectorius*, during this study. This species binds much of the soil on the high coastal cliffs to the island without *Pandanus tectorius* stands the threat of large-scale soil and natural vegetation erosion looms large.

The small populations of species poor remnant native vegetation on the island, many of which have loss their ability to disperse throughout the island, which in turn leaves the vegetation even more

vulnerable to the effects of invasive species invasion as they totally lack the competitive ability to deal with invaders. Conservation management and restoration on Pitcairn will have to play an even more increasing role in both maintaining and restoring the damaged ecosystem on Pitcairn Island.

Species conservation work on the island during this project highlighted the often cited "crisis discipline" nature of species conservation with environmental stochastic threats being of most concern. Island species appear to be capable of maintaining high levels of diversity with extremely low population numbers. The use of island theory to deal with increasing habitat fragmentation in continental areas as often been cited as useful. However, the main difference between island populations and reduced continental populations is that island species have most likely have always exited in low populations, they have already been through bottlenecks and have often shed deleterious alleles and thus are adapted to living in small populations in "island" conditions. Continental plant populations however, are not used to existing in such low numbers and thus are not adapted genetically or in any other way for dealing with small "island" habitats. The comparisons between species, which survive in habitat fragments on continents, and island plant populations is misleading as the two cannot be compared.

Pitcairn island is entering a phase of economic development, while this development is occurring later on Pitcairn than other Polynesian islands and the lessons learnt with regard to the detrimental effects of excessive tourism leading to habitat destruction are well known for islands. However, Pitcairn unlike other islands, which have developed many years previous, is not starting from an environmentally pristine state. The existing habitat fragmentation on the island, the critically low endemic species numbers, and the number of invasive species already existing on the island has already caused much environmental degradation. Any new development if not acutely environmentally aware will destroy the remaining "natural" environment on the island very quickly. Integrating the development of the island in conjunction with the local community and with increased management of the remaining unique habitats and species and setting out a long-term plan of action for dealing with invasive species is required, if the island is to become economically, socially and ecologically self- sufficient for the future.

Bibliography

Aide, T.M., Zimmerman, J.K., Pascarella, J.B. Rivera, L. Marcano-Vega, H. 2000. Forest regeneration in a chronosequence of tropical abandoned pastures: implications for restoration ecology. *Restoration Ecology* **8**(4):328-340.

Allen, S.E. 1989. Chemical analysis of Ecological Materials. Oxford. Blackwell Scientific Publications

Allendorf, F.W. & Lunquist, L.L. 2003. Population biology, evolution, and control of invasive species. *Conservation Biology* **17**(1):24-30

Amos, W. & Balmford, A. 2001. When does conservation genetics matter? *Heredity* **87**: 257-265.

Arencibia, A.D., Carmona, E., Perez, G., Vinagre, F., Hemerly, A.S., Santana, I. 2005. Identification and characterization of hypervariable sequences within the *Saccharum* complex. *Plant Science* **169**:478-486.

Ashby, W.C. 1987. Restoration ecology: a synthetic approach to ecological research. In: W.R. Jordan, M.E. Gilpin & J.D. Aber (eds.), pp.342. Restoration Ecology: a Synthetic Approach to Ecological Restoration. Cambridge University Press, Cambridge.

Augspurger, C.A. 1984. Seedling survival of tropical tree species:interactions of dispersal, distance, light gaps and pathogens. *Ecology* **65**:1705-1712

Augusto, L., Dupouet, J., Picard, J. & Ranger, J. 2001. Potential contribution of the seed bank in coniferous plantations to the restoration of native deciduous forest vegetation. *Acta Oecologica* **22**:470-479

Avise, J.C. 1994. Molecular markers, Natural History and Evolution. Chapman & Hall.

Ayres, D.R. & Ryan, F.J. 1997. The clonal and population structure of a rare endemic plant *Wyethia reticulata* (Asteraceae): allozyme and RAPD analysis. *Molecular Ecology* **6**:761-772.

Bahn, P. & Flenley, J.R. 1992. Easter Island. Earth Island. Thames and Hudson, London.

Baker, H.G. 1955. Self-incompatibility and establishment after long-distance dispersal. *Evolution* **9**:347-349

Bakker, J.P., Poschlod, P., Strykstra, R.J., Bekker, R.M. & Thompson, K. 1996. Seed banks and seed dispersal: important topics in restoration ecology. *Acta Botanica Neerlandia* **45**(4):461-490.

Barrett, S.C.H. & Kohn, J.R. 1991. Genetics and evolutionary consequences of small population sizes in plants: implications for conservation. In: D.A. Falk & K.E. Holsinger (eds.), pp.31-44. Genetics and Conservation of Rare Plants. Oxford University Press.

Barton, N.H. 1989. Founder effect speciation. In: D. Otte and J.A. Endler (eds.), pp. 229-256. Speciation and its Consequences. Sinauer, Sunderland, M.A.

Baum, D. 1992. Phylogenetic species concepts. *Trends in Ecology and Evolution* **7**:1-3.

Bell, B.D. & Bell, E. 1998. Habitat Restoration: Pitcairn Islands, South Pacific – Eradication of rats and feral cats, April to December 1997. Wildlife Management International Ltd. A Report for the Foreign & Commonwealth Office and the Pitcairn Islands Administration.

Bensch, S & Åkesson, M. 2005. Ten years of AFLP in ecology and evolution: why so few animals? *Molecular Ecology* **14**: 2899-2914.

Benton,T.G. & Spencer, T. (eds.) 1995. The Pitcairn Islands: Biogeography, Ecology and Prehistory. Academic Press. London.

Bergstrom, D.M. & Chown, S.L. 1999. Life at the front: history, ecology and change on southern ocean islands. *Trends in Ecology and Evolution* **14**(2): 472- 477.

Berry, R.J. 1971. Conservation aspects of the genetical constitution of populations. In: E. Duffey & A.S. Watt (eds.), pp.177-206. The Scientific Management of Animal and Plant Communities for Conservation, Symposium of the British Ecological Society, II, Blackwell Scientific Publications, Oxford

Berry, R.J. 1992. The significance of island biotas. *Biological Journal of the Linnaean Society* **46**: 3-12

Binggeli, P., Hall, J.B. & Healey, J.R. 1998. An Overview of Invasive Woody Plants in the Tropics. Bangor, Wales: School of Agricultural and forest Sciences.

Blossey, B. & Nötzold, R. 1995. Evolution of increased competitive ability in invasive non-indigenous plants: a hypothesis. *Journal of Ecology* **83**:887-889.

Born, W., Rauschmayer, F., Brauer, I. 2005. Economic evaluation of biological invasions-a survey. *Ecological Economics* **55**:321-336.

Bowles, M.L. & Apfelbaum, S.I. 1989. Effects of land use and stochastic events on heart plantain (*Plantago cordata* Lam) in an Illinois stream system. *Natural Areas Journal* **9**:90-107.

Bradshaw, A.D. 1997. What do we mean by restoration? In: K.M. Urbanska, N. R. Webb & P. J. Edwards (eds.), pp 8-14. Restoration Ecology and Sustainable Development. Cambridge University Press.

Brockie, R.E., Loope, L.L., Usher, M.B. & Hamann, O. 1988. Biological invasions of island nature reserves. *Biological Conservation* **44**:9-36.

Brokaw, N.V.L. 1985. Treefalls, regrowth, and community structure in tropical forests. In: S.T.A. Pickett and P.S. White, (eds.), pp. 53-69. The Ecology of Natural Disturbance and Patch Dynamics, Academic Press, San Diego.

Brooke, M de L., Hepburn, I. & Trevelyan, R.J. 2004. Henderson Island; World Heritage Site; Management Plan 2004-2009. Foreign & Commonwealth Office, London, Pitcairn Islands Administration & Royal Society for the Protection of Birds.

Brooks, T.M., Mittermeier, A., Mitteermeier, C.G., Da Fonseca, G.A.B., Rylands, A.B., Konstant, W.R., Flick, P., Pilgrim, J., Oldfield, S., Magin,G., Hilton-Taylor, C. 2002. Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology* 1694:909-923.

Brown, A.H.D. & Briggs, J.D. 1991. Sampling strategies for genetic variation in ex-situ collections of endangered plant species. In: D.A. Falk & K.E. Holsinger (eds.), pp99-119. Genetics and Conservation of Rare Plants, Oxford University Press.

Brown, K.A. & Gurevitch, J. 2004. Long-term impacts of logging on forest diversity in Madagascar. *Proceedings of the National Academy of Sciences USA*. **101**(16): 6045–6049.

Brown, K.A., Scatena, F.N., Gurevitch, J. 2006. Effects an invasive tree on community structure and diversity in a tropical forest in Puerto Rico. *Forest Ecology and Management* **226** (1-3):145-152.

Bruford, M.W.O., Hanotte, J.E.Y., Brookfield, J.E. & Burke, T. 1992. Single locus and multilocus DNA fingerprinting. In: C. A. R. Hoelzel (ed.), pp. 225-259. Molecular Genetics Analysis of Populations. Oxford University Press, New York.

Brussard, P.F. 1991. The role of ecology in biological conservation. *Ecological Applications* **1**:6-12

Buhle, E.R., Margolis, M., Ruesink, J.L. 2005. Bang for buck, cost-effective control of invasive species with different life histories. *Ecological Economics* **52**:355-366.

Burgman, M.A., Akcakaya, H.R., & Loew, S.S. 1988. The use of extinction models for species conservation. *Biological Conservation* **43**:9–25.

Bush, M.B. 1996. Amazonian conservation in a changing world. *Biological Conservation* **76**:219-228.

Cabin, R.J., Wellers, S., Lorence, D., Hadway, C. 1999. Restoring tropical dry forests with direct seeding: the effects of light water and weeding (Hawaii). *Ecological Restoration* **17**:237-238.

Caesar, A. 2005. Melding ecology, classical weed biocontrol and plant microbial ecology can inform improved practices in controlling invasive plant species. *Biological Control* **35**:240-246.

Camacho-Cruz A., González-Espinosa M., Wolf J.H.D. and De Jong B.H.J. 2000. Germination and survival of tree species in disturbed forests of the highlands of Chiapas, Mexico. *Canadian Journal of Botany* **78**:1309–1318.

Campano-Camargo, J.L., Kossman-Feraz, I.D. Imakawa, A.M. 2002. Rehabilitation of Degraded Areas of Central Amazonia, Using Direct Sowing of Forest Tree Seeds. *Restoration Ecology* **10**(4):636–644.

Casgrain, P. D. 1999. Principal Coordinates Analysis Version 4.0. Départment de Sciences Biologiques, Université de Montreal, Québec.

Chao, C-C., Devanand, P.S. & Chen, J. 2005. AFLP analysis of genetic relationships among *Calathea* species and cultivars. *Plant Science* **168**:1459-1469.

Chapman, CA & Chapman, L.J. 1999. Forest restoration in abandoned agricultural land. A case study from east Africa. *Conservation Biology* **13**:1301-1311.

Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**:237-268.

Chase, M.W. & Hills, H.H. 1991. Silica gel: An ideal material for field preservation of leaf samples for DNA studies. *Taxon* **40**: 215-220.

Chase, M.W., Soltis, D.E., Olmsted, R.G., Morgan, D., Les, D.H., Mishler, B.D., Duvall, M.R., Price, R.A., Hills, H.G., Qiu, Y-L., Kron, K.A., Rettig, J.H., Conti, E., Palmer, J.D., Manhart, J.R., Sytsma, K.J., Michaels, H.J., Kress, W.J., Karol, K.G., Clark, W.D., Hedren, M., Gaut, B.S., Jansen, R.K., Kim, K-J., Wimpee, C.F., Smith, J.F., Furnier, G.R., Strauss, S.H., Xiang, Q-Y., Plunkett, G.M., Soltis, P.S., Swensen, S.M., Williams, S.E., Williams, S.E., Gadek, P.A., Quinn, C.J., Eguiarte, L.E., Golenberg, E., Learn, G.H.Jr., Graham, S.W., Barrett, S.C.H., Dayanandan, S., Albert, V.A. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. *Annals of the Missouri Botanical Garden* **80**: 528-580.

Ciprianai, G., Testolin, R. & Gardner, R. 1998. Restriction site variation of PCR-amplified chloroplast DNA regions and its implication for the evolution and taxonomy of *Actinidia*. *Theoretical and Applied Genetics* **96**:389-396.

Clark, B. & Grant, P.R. (eds.) 1996. Evolution on Islands. *Philosophical Transactions of the Royal Society of London*, B. **351**:723-854.

Clark, M.J. 1997. Ecological restoration - the magnitude of the challenge: an outsider's view. In: K.M. Urbanska, N.R. Webb & P.J. Edwards (eds.), pp. 397. Restoration Ecology and Sustainable Development. Cambridge University Press, Cambridge.

Clout, M.N. & Veitch, C.R.2002 (eds). Turning the tide: the eradication of invasive species pp. 336-341. IUCN SSC Invasive species specialist group. IUCN, Gland, Switzerland and Cambridge, U.K

Coart, E., Vekemans, X., Smulders, M.J.M., Wagner, I., Van Huylendroeck, J., Van Bockstaele, E., & Roldán-Ruiz, I. 2003. Genetic variation in the endangered wild apple (*Malus sylvestris* (L.) Mill.) in Belgium as revealed by amplified fragment length polymorphism and microsatellite markers. *Molecular Ecology* **12**:845-857.

Coates DJ. 1992. Genetic consequences of a bottleneck and spatial genetics structure in the triggerplant *Stylidium coroniforme* (Stylidiaceae). *Heredity* **69**:512-520.

Coblentz, B. E. 1990. Exotic organisms: a dilemma for conservation biology. *Conservation Biology* **4**(3): 261-265.

Cochran, W.G. 1951. Testing a linear relation between variances. *Biometrics* **7**:17-32.

Cohen, S. Braham, R. & Sanchez, F. 2004. Seedbank viability in disturbed longleaf pine sites. *Restoration Ecology* **12** (4): 503-515.

Crawley, M.J. 1987. What makes a community invasible? In: A.J. Gray, M.J. Crawley & P.J. Edwards (eds.), pp.429-453. Colonization, Succession and Stability. Blackwell, Oxford.

Cronk Q.C.B. 1997. Islands: stability, diversity, conservation. *Biodiversity and Conservation* **6**:477-493.

Cronk, Q.B.C. & Fuller, J.L. 2001. Plant Invaders: The Threat to Natural Ecosystems. WWF Earthscan Publications Ltd.

Cronk, Q.B.C. 1989. The past and present vegetation of St. Helena. *Jounral of Biogeography* **16**:47-64.

Cross, J.R. 1981. The establishment of *Rhododendron ponticum* in Killarney oakwoods, S.W. Ireland. *Journal of Ecology* **69**:807-824.

Csurhes, S. & Edwards, R. 1998. Potential environmental weeds in Australia: Candidate Species for Preventative Control. Queensland Department of Natural Resources, Australia.

Curtis, T.G.F & McGough, H.N. 1987. The Irish Red Data Book. I Vascular Plants. Wildlife Service, Office of Public Works, Dublin.

Daehler, C.C., Denslow, J.S., Ansari, S., Kuo, H-C. 2003. A risk assessment system for screening out invasive pest plants from Hawaii and other pacific islands. *Conservation Biology* **18**(4):360-368.

De Souza & Batista 2004. Restoration of seasonal semi deciduous forests in Brazil: influence of age and restoration design on forest structure. *Forest Ecology and Management* **191**(1-3):185-200.

Dehnen-Schmutz, K., Perrings, C. & Williamson, M. 2004. Controlling *Rhododendron ponticum* in the British Isles: an economic analysis, *Journal of Environmental Management* **70**:323–332.

DeJong, T.M. 1975. A comparison of three diversity indices based on their components of richness and evenness. *Oikos* **26**:222-227.

Demel, T. & Granström, A. 1995. Soil seed banks in dry afro-montane forests of Ethiopia. *Journal of Vegetation Science* **6**:777-786.

Diamond, J. 2006. Past Societies. In: Collapse: How Societies Choose or Fail to Survive. pp.77-120. Penguin Books.

DiTomaso, J.M. 2000. Invasive weeds in rangelands: Species, impacts and management. *Weed Science* **48**:255-265.

Dixon, I. R; K. W. Dixon, and M. Barrett, 2002. Eradication of buffel grass (*Cenchrus ciliaris*) on Airlie Island, Pilbara Coast, Western Australia. In: C. R. Veitch & M.N. Clout (eds), pp: 374-380. Turning the Tide: the Eradication of Invasive Species. IUCN SSC Invasive Species Specialist Group. IUCN. Gland. Switzerland and Cambridge. UK.

Dowe, J.L. Benzie, J. & Ballment, E. 1997. Ecology and Genetics of *Carpoxylon macrospermum* H. Wendl. & Drude (Arecaceae), an endangered palm from Vanuatu. *Biological Conservation* **79**:205-216.

Drake, D.R. 1998. Relationships among seed rain, seed bank, and vegetation of a Hawaiian forest. *Journal of Vegetation Science* **9**:815-828.

Drummond, R.S.M., Keeling, D.J., Richardson, T.E., Gardner, R.C. & Wright, S.D. 2000. Genetic analysis and conservation of 31 surviving individuals of a rare new Zealand tree, *Meterosideros bartlettii* (Myrtaceae). *Molecular Ecology* **9**:1149-1157.

Dyer A.R., Rice K.J. 1999. Effects of competition on resource availability and growth of a California bunchgrass. *Ecology* **80**:2697–2710.

Ehrendofer, E. 1979. Reproductive biology in island plants. In: D. Bramwell (eds.), pp.293-306. Plants and Islands. Academic Press, London.

Elliott, S., Blakesley, D., Maxwell, J.F., Doust, S., Suwannaratana, S. 2006. How to plant a forest: the principles and practice of restoring tropical forests. The Forest Restoration Research Unit, Chang Mai University, Chang Mai, Thailand.

Ellsworth, J.W., Harrington, R.A. & Fownes, J. 2004. Seedling emergence, growth and allocation of oriental bittersweet: effects of seed input, seed bank, and forest floor litter. Forest Ecology and Management 190:255-264.

Elton C.S. 1958. The Ecology of Invasions by Animals and Plants. Methuen, London.

Environment Australia 1997. Norfolk Island Weed Control Manual for Selected Weeds Occuring in Norfolk Island National Park. Commonwealth of Australia © Environment Australia, Parks Australia (South), Norfolk Island, P.O. Box 310, Norlk Island, South Pacific 2899.

Erfmeier, A. & Bruelheide, H. 2004. Comparisons of native and invasive *Rhododendron ponticum* populations: Growth, reproduction and morphology under field conditions. *Flora* **199**:120-133.

Ewell, J.J., D.J. O'Dowd, J. Bergelson, C.C. Daehler, C.M. D'Antonio, L.D. Gomez, D.R. Gordon, R.J. Hobbs, A. Holt, K.R. Hopper, C.E. Hughes, M. LaHart, R.R.B. Leakey, W.G. Wong, L.L. Loope, D.H. Lorence, S.M. Louda, A.E. Lugo, P.B. McEvoy, D.M. Richardson, and P.M. Vitousek 1999. Deliberate introductions of species: research needs - benefits can be reaped, but risks are high. *Bioscience* **49**:619-630.

Felsenstein, J. 1985. Confidence limits oh phylogenies: an approach using bootstrap. *Evolution* **39**:783-791.

Feynman, R. 1980. The Character of Physical Law. MIT Press, Cambridge Massachussetts, p. 34.

Fiedler, P.L. & Jain, S.K. (eds.) 1992. Conservation Biology: the Theory and Practice of Nature Conservation, Preservation and Management. Chapman & Hall, London.

Fiedler, P.L. & Karieva, P.M. (eds.) 1998. Conservation Biology for the Coming Decade 2nd Edition. Chapman & Hall. International Thomson Publishing. New York.

Flint, E. & Rehkemper, C. 2002 Control and eradication of the introduced grass, *Cenchrus echinatus*, at Laysan island, Central Pacific Ocean. In Veitch, C.R & Clout, M.N. (eds). Turning the Tide: the Eradication of Invasive Species pp. 336-341. IUCN SSC Invasive Species Specialist Group. IUCN, Gland, Switzerland and Cambridge, U.K.

Florence, J., Waldren, S., Chepstow Lusty, A.J. 1995. The flora of Pitcairn Islands- a review. *Biological Journal of the Linnaean Society* **56** (1-2):79-119.

Forrest, A.D., Hollinsworth, M.L., Hollingsworth, P.M., Sydes, C. & Bateman, R.M. 2004. Population genetic structure in European populations of *Spiranthes romanzoffiana* set in the context of other genetic studies on orchids. *Heredity* **92**: 218-227.

Fosberg, F. R., Sachet, M-H., Oliver, R.L. 1979. A geographical checklist of the Micronesian Dicotyledonae. *Micronesia* **15**:1-295.

Fosberg, F.R. 1991. Polynesian plant environments. In P.A. Cox & S.A. (eds.), pp.11-23 Islands, Plants and Polynesians: an Introduction to Polynesian Ethnobotany Portland: Dioscorides Press.

Fosberg, F.R., Paulay, G., Spenser, T., & Oliver, R. 1989. New collections and notes on the plants of Henderson, Pitcairn, Oeno and Ducie Islands. *Atoll Research Bulletin* **329**:1-18.

Fosberg, F.R., Sachet, M-H. & Stoddart, D.R. 1983. Henderson Island (South-Eastern Polynesia): a summary of current knowledge. *Atoll Research Bulletin* **272**: 1-47.

Foster, R.D. & Wetzel, P.R. 2005. Invading monotypic stands of *Phalaris arundinacea*: A test of fire, herbicide and woody and herbaceous native plant groups. *Restoration Ecology* **13**(2):318-324.

Fowler, J., Cohen, L. & Jarvis, P. 1998. Practical Statistics for Field Biology 2nd Ed. John Wiley & Sons Ltd., The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England.

Francisco-Ortega, J., Santos-Guerra, A., Kim, S.C. & Crawford, D.J. 2000. Plant genetic diversity in the Canary Islands: a conservation perspective. *American Journal of Botany* **87**:909–919.

Frankel, O.H. & Soulé, M.E. 1981. Conservation and Evolution. Cambridge University Press, Cambridge, U.K.

Frankham, R. 1998. Inbreeding and extinction: Island populations. *Conservation Biology* **12**(3):665-675.

Franz, C.M. & Keiffer. C.H. 2000. The effectiveness of the EZJECT capsule injection system against the invasive shrub, Amur Honeysuckle. *The Ohio Woodland Journal* **7**:19-20.

Fritts, T.H. & Rodda, G.H. 1998. The role of introduced species in the degradation of island ecosystems: a case history of Guam. *Annual Review of Ecology & Systematics* **29**:113-140.

Gardner, R. 1998. Restriction-site variation of PCR-amplified chloroplast DNA regions and its implication for the evolution and taxonomy of *Actinidia*. *Theoretical Applications in Genetics* **96**:389-396.

Garwood, N.C. 1989. Tropical seed banks: a review. In: M.A. Leck, V.T. Parker & R.L. Simpson (eds.), pp. 149-209. Ecology of Soil Seed Banks. Academic Press, San Diego, C.A.

Gaudeul, M., Taberlet, P. & Till-Bottraud, I. 2000. Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L. (Apiaceae), inferred from amplified fragment polymorphism markers. *Molecular Ecology* **9**: 1625-1637.

Gene Codes Corporation. 1998. SEQUENCHER © 775 Technology Drive, Suite 100A, Ann Arbor, MI 48108. USA.

Gerlach, J. 2004. A 10 year study of changes in forest vegetation on Silhouette Island, Seychelles. *Journal for Nature Conservation* **12**: 149-155.

Ghisalberti, E. 2000. Lantana camara L.(Verbenaceae). *Fitoterapia* **71**:467-486.

Gielly,L., & Taberlet,P. 1994.The use of chloroplast DNA to resolve plant phylogenies: non-coding versus rbcL sequences. *Molecular Biology and Evolution* **11**:769-777.

Gilbert, O.L. & Anderson, P. 2005. Habitat Creation and Repair. Oxford University Press, Oxford.

Given, D.R. 1993. Changing aspects of endemism and endangerment in Pteridophyta. *Journal of Biogeography* **20**:293-302.

Given, D.R. 1994. The Principles and Practices of Plant Conservation. London: Chapman & Hall.

Given, D. R. 1992. An Overview of the Terrestrial Biodiversity of Pacific Islands. Report, South Pacific Regional Environment Programme, Apia, Western Samoa.

Godefroid, S., Phartyal, S.S., Weyembergh, G. & Koedam, N. 2005. Ecological factors controlling the abundance of non-native invasive black cherry (*Prunus serotina*) in deciduous forest understory in Belgium. *Forest Ecology & Management* **210**:91-105.

Godt, M.J.W. & Hamrick, J.L. 1999. Population genetic analysis of *Elliottia racemosa* (Ericaceae) a rare Georgia shrub. *Molecular Ecology* **8** (1): 75-82.

Goodman, D. 1987. The demography of chance extinction. In: M.E. Soulé (ed.), pp.59-68 Viable Populations for Conservation. Cambridge: Cambridge University Press.

Göthesson, L-Å. 1997. Plants of the Pitcairn Islands, including local names and uses. Sydney: University of New South Wales.

Grimshaw, H.M. 1989. Analysis of soil. In: S.E. Allen (ed.) pp.7-45. Chemical Analysis of Ecological Materials. Oxford. Blackwell Scientific Publications.

Groombridge, B. & Jenkins, M.D. 2002. World Atlas of Biodiversity. Earth's Living Resources in the 21st Century. United Nations Environment Programme-World Conservation Monitoring Centre, Univeristy of California Press, Berkeley.

Groove, R.H. 1995. Green Imperialism: Colonial Expansion, Tropical Island Edens, and the Origins of Environmentalism 1600-1860 pp.24. Cambridge University Press.

Groves, F.J. Kruger, M. Rejmánek & M. Williamson (eds). Biological Invasions- a Global Perspective. Wiley New York.

Guedes, C.M., Pinto, A.B., Moreira, R.F.A. & de Maria, C.A.B. 2004. Study of the aroma compounds of roseapple (*Syzygium jambos*) fruits from Brazil. *European Food Research and Technology* **211**(5):1438-2377.

Guerrant, E.O. & Pavlik, B.M. 1998. Reintroduction of rare plants: genetics, demography and the role of ex-situ conservation methods. In: P.L. Fiedler & P.M. Kareiva (eds.), pp80-108. Conservation Biology for the Coming Decade 2nd Edition. Chapman and Hall. International Thompson Publishing.

Gurevitch, J & Padilla. D.K. 2004. Are invasive species a major cause of extinction? *Trends in Ecology and Evolution* **19**(9):470-474.

Hamann, O. 1984. Changes and threats to the vegetation. In: R. Perry (ed.) pp115-132. Key Environments. Galápagos. Pergamon Press, Oxford, U.K.

Hamann, O. 1979. Regeneration of vegetation on Sante Fe and Pinta Island, Galápagos, after the eradication of goats. *Biological Conservation* **15**:215-236.

Hamrick , J.L., Godt, M.J.W. , Murawski, D.A. & Loveless, M.D. 1991. Correlations between species traits and allozyme diversity: implication for conservation biology. In: D.A. Falk & K.E. Holsinger (eds.), pp. 75-86. Genetics and Conservation of Rare Plants. Oxford University Press.

Harbourne, M. 2004. The Characterisation of Genetic Diversity of Common Ash (*Fraxinus excelsior* L.) in Ireland and around Europe. PhD. Thesis. University of Dublin, Trinity College.

Harrison, A.F. & Bocock, K.L. 1981. Estimation of soil bulk density from loss on ignition values. *Journal of Ecology* **18**:919-927.

Hartley, S. & Kunin, W.E. 2003. Scale dependency of rarity, extinction risk and conservation priority. *Conservation Biology* **17**(6):1559-1570.

Hartman, K.M. & McCarthy, B.C. 2004. Restoration of a forest understory after removal of an invasive shrub, Amur Honeysuckle (*Lonicera maackiii*). *Restoration Ecology* **12**(2):154-165.

Heads, M.J. 1996. Biogeography, taxonomy and evolution in the Pacific genus *Coprosma* (Rubiaceae). *Candollea* **51**:381-405.

Heschel, M.S. & Paige, K.N. 1995. Inbreeding depression, environmental stress and population size variation in Scarlet gilia (*Ipomopsis aggregata*). *Conservation Biology* **9**: 126-133.

Hess, A.L. 1990. Overview: Sustainable development and environmental management of small islands. In: W. Beller, P. d'Ayala, P. Hein, pp. 3-33. Sustainable Development and Environmental Management of Small Islands. Man and the Biosphere Series Volume 5. UNESCO Paris.

Higgs, E.S. 1997. What is good ecological restoration? *Conservation Biology* **11**:338-348.

Hill, M.O. & Gausch, H.G. 1980. Detrended correspondence analysis, an improved ordination technique. *Vegetatio* **42**:47-58.

Hodkinson, T.R., Chase, M.W. & Renvoize, S.A. 2002. Characterisation of a genetic resource collection for *Miscanthus* (Saccharinae, Andropogoneae, Poaceae) using AFLP and ISSR PCR. *Annals of Botany* **89**:627-636.

Hodkinson, T.R., Renvoize, S.A.,Ni Chonghaile,G., Stapelton, C.M.A., & Chase, M. 2000. A comparison of ITS Nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae) *Journal of Plant Research* **113**:259-269.

Holl, K.D., Loik, M.E., Lin, E.H.V. & Samuels, I.A. 2000. Tropical forest restoration in abandoned pastures in Costa Rica: obstacles and opportunities. *Restoration Ecology* **8**:339-349.

Holmes, P.A. & Cowling, R.M. 1997. Diversity, composition and guild structure relationships between soil-stored seed bans and mature vegetation in alien plant-invaded South African fynbos scrublands. *Plant Ecology* **133**:107-122.

Holmes, P.M., Richardson, D.M. Van Wilgen, B.W. & Gelderbloom, C. 2000. Recovery of South African fynbos vegetation following alien woody plant clearing and fire: implications for restoration. *Austral Ecology* **25**:631-639.

Holsinger, K.E., Lewis, P.O. & Dey, D.K. 2002. A Bayesian approach to inferring population structure from dominant markers. *Molecular Ecology* **11**:1157-1164.

Hoot, S.B., Culham, A., & Crane, P.R. 1995. The utility of atpB gene sequences in resolving phylogenetic relationships: Comparison with rbcL and 18S ribosomal DNA sequences in the Lardizabalaceae. *Annals of the Missouri Botanical Garden* **82**:194-208.

Horvitz, C.C. & Schemske, D.W. 1998. Seed dispersal and environment heterogeneity in a neotropical herb: a model of population and patch dynamics. In: A. Estrada & T.H. Fleming (eds.), pp.169-186.Frugivores and Seed Dispersal. Dr. W. Junk, Dordrecht, The Netherlands.

Horvitz, C.C., Pascarella, J.B., McMann, S., Freedman, A., Hofstetter, R.H. 1998. Functional roles of invasive non-indigenous plants in hurricane-affected subtropical hardwood forests. *Ecological Applications* **8**: 947-974.

Howe, H.F. 1999. Response of *Zizia aurea* to seasonal mowing and fire in a restored prairie. *American Midland Naturalist* **141**(2): 373-380.

Howell, E.A. 1986. Woodland restoration an overview. *Restoration and Management Notes* **4**:13-17.

Hunter, Jr. M.L. 1996. Fundamentals of Conservation Biology. Blackwell Science, Cambridge, Massachussetts.

IUCN 2001. IUCN Red list categories. IUCN Species Survival Commission, Kew. (http://www.iucnredlist.org/info/categories_criteria2001 accessed 4th March 08).

IUCN 2000. Guidelines for the prevention of biodiversity loss caused by alien invasive species (http://www.iucn.org/themes/ssc/publications/policy/invasivesEng.htm
accessed 4th March 08).

Jarne, P. & Lagoda, P.J.L. 1996. Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* **11**(10):424-429.

Jeffrey, D.W. 1970. A note on the use of ignition loss as a means for the approximate estimation of soil bulk density. *Journal of Ecology* **58**: 297-299.

Johansson, T. 1985. Herbicide injections into stumps of aspen and birch to prevent regrowth. *Weed Research* **25**: 39:45.

Johnson, L.A.S., & Briggs. 1984. Myrtales & Myrtaceae-a phylogenetic analysis. *Annals of the Missouri Botanic Gardens* **71**:700-765.

Jordan, W.R., Gilpin, M.E., & Aber, J.D.1987. Restoration Ecology: ecological restoration as a technique for basic research. In: W.R. Jordan, M.E. Gilpin & J.D (eds.), pp.342. Restoration Ecology: a Synthetic Approach to Ecological Research. Cambridge University Press, Cambridge.

Juan, A., Crespo, B., Cowan, R.S., Lexer, C. & Fay, M.F. 2004. Patterns of variability and gene flow in *Medicago citrina*, an endangered endemic of islands in the western Mediterranean, as revealed by amplified fragment length polymorphism (AFLP). *Molecular Ecology* **13**: 2679-2690.

Jules, E.S. 1998. Habitat fragmentation and demographic change for a common plant: *Trillium* in old-growth forest. *Ecology* **79**:1645-1656.

Kajita, T., Kamiya, K., Nakamura, K., Tachida, H., Wickneswari, R., Tsumura, Y., Yoshimaru, H., and Yamazaki, T. 1998. Molecular Phylogeny of Dipterocarpaceae in Southeast Asia based on Nucleotide Sequences of matK, trnL intron, and trnL-trnF intergenic spacer region in chloroplast DNA. *Molecular Phylogeny and Evolution* **10**:202–209.

Karp, A., Seberg, O. & Buiatti, M. 1996. Molecular techniques in the assessment of botanical diversity. *Annals of Botany* **78**: 43-149.

Kastadalen, A. 1982. Changes in the biology of Santa Cruz Island between 1935 and 1965. *Noticias de Galápagos* **35**:7-12.

Kay, Q & John, R. 1997. Patterns of variation in relation to the conservation of rare and declining plant species. In: T.E. Tew. T.J. Crawford, J.W. Spenser, D.P. Stevens, M.B. Usher & J. Warren (eds.), pp.41-55. The Role of Genetics in Conserving Small Populations. Peterborough, JNCC.

Keane, R.M. & Crawley, M.J. 2003. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* **17**(4):164-170.

Keiper, F.J. & McConchie, R. 2000. An analysis of genetic variation in natural populations of *Sticherus flabellatus* [R. Br. (St. John)] using amplified fragment length polymorphism (AFLP) markers. *Molecular Ecology* **9**: 571-581.

Keith, D.A. 1998. An Evaluation and Modification of World Conservation Union Red List Criteria for Classification of Extinction Risk in Vascular Plants. *Conservation Biology* **12**: 1076-1090.

Keith, D.A. 2000. Sampling designs, field techniques and analytical methods for systematic population surveys. *Ecological Management and Restoration* **1**(2):125-139.

Kent, M & Coker, P. 1992. Vegetation Description and Analysis a Practical Approach. pp1-2. Belhaven Press, London.

King, L.M. & Schaal, B.A. 1989. Ribosomal-DNA variation and distribution in Rudbeckia missouriensis. *Evolution* **43**:1117-1119.

Kingston, N. & Waldren, S. 2003. The plant communities and environmental gradients of Pitcairn Island: The significance of invasive species and the need for conservation management. *Annals of Botany* **92** (1):31-40.

Kingston, N. & Waldren, S. 2005. A conservation appraisal of the rare and endemic vascular plants of Pitcairn Island. *Biodiversity and Conservation* **14**:781-800.

Kingston, N. 2001. The flora and Vegetation of Pitcairn island - its phytogeography and conservation. PhD. Thesis. University of Dublin, Trinity College.

Kingston, N., Waldren, S. & Bradley, U. 2003. The phytogeographical affinities of the Pitcairn Islands - a model for South-Eastern Polynesia? *Journal of Biogeography* **30**:1311-1328.

Kingston, N., Waldren, S. & Smyth, N. 2004. Conservation genetics and ecology of *Angiopteris chauliodonta* Copel. (Marattiaceae), a critically endangered fern from Pitcairn Island, South Central Pacific Ocean. *Biological Conservation* **117** (3):309-319.

Klinkhamer, P.G.L., De Jong, T.J. Metz, J.A.J. & Val, J. 1987. Life history tactics of annual organisms: the joint effects of dispersal and delayed germination. *Theoretical Population Biology* **32**:127-156.

Knapp, E.E. and K.J. Rice. 1994. Starting from seed: genetic issues in using native grasses for restoration. *Restoration & Management Notes* **12**:1: 40-45.

Kochummen, K.M. 1995. *Eugenia* In: F.S.P. Ng (ed.), *Tree Flora of Malaya* **3**:172-247. Longman, London. Reprint of 1978 edition.

Kourtev, P.S., Ehrenfeld, J.G., Haggblom, M. 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biology & Biochemistry* **35**(7):895-905.

Krauss, S.L. 2000. Accurate gene diversity estimates from amplified fragment length polymorphism (AFLP) markers. *Molecular Ecology* **9**:1241-1245.

Krauss, S.L. 1999. Complete exclusion of nonsires in an analysis of paternity in a natural plant population using amplified fragment length polymorphism (AFLP) *Molecular Ecology* **8** (2):217-226.

Krauss, S.L., Dixon, B., Dixon, K.W. 2002. Rapid genetic decline in a translocated population of the endangered plant *Grevillea scapigera*. *Conservation Biology* **16** (4):986-994.

Lai, P.C.C. & Wong, B.S. 2005. Effects of tree guards and weed mats on the establishment of native tree seedlings: implications for forest restoration in Hong Kong, China. *Restoration Ecology* **13**(1):138-143.

Lake, J.C. & Leishman, M.R. 2004. Invasion success of exotic plants in natural ecosystems: the role of disturbance, plant attributes and freedom from herbivores. *Biological Conservation* **12**:215-226.

Lambeck, R.J. 1997. Focal species: a multi-species umbrella for nature conservation. *Conservation Biology* **11**:849-856.

Lambeck, R.J. 1999. Landscape planning for biodiversity conservation in agricultural regions: a case study from the wheatbelt of Western Australia. Biodiversity Technical Paper 2. Environment Australia, Canberra, Australia.

Lande, R. & Barrowclough, G.F. 1987. Effective population size, genetic variation, and their use in population management. In: M.E. Soulé (eds.), pp 87-123. Viable Populations for Conservation. Cambridge University Press, Cambridge.

Lande, R. 1988. Genetics and Demography in Biological Conservation. *Science* **241**:1455-1460.

Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. *American Naturalist* **142**(6):911-927.

Lande, R. 1998. Anthropogenic, ecological and genetic factors in extinction and conservation. *Researches on Population Ecology* **40**:259-269.

Lawesson, J.E. 1990. Alien plants in the Galápagos Islands, a summary. In: J.E. Lawesson, O.Hamann, G.Rodgers and H.Ochoa (eds.). Botanical Research and Management in Galápagos. *Monograph in Systematic Botany* **32**:15-20 Missouri Botanical Garden, St. Louis.

Lawton, J.H. & May. R.M. (ed.) 1995. Extinction rates. Oxford University Press, Oxford.

Lee, W.G., Weatherall, I.L. & Bastow-Wilson, J.1994. Fruit conspicuousness in some New Zealand *Coprosma* (Rubiaceae) species. *Oikos* **69**:87-94.

Legendre, P. & Legendre, L. 1998. Numerical Ecology 2nd Edition. Developments in Environmental Modelling. Amsterdam:Elsevier.

Lemmens, R.H.M.J. 1995. *Syzygium* Gaertner. In: R.H.M.J. Lemmens, I. Soerianegara & W.C. Wong (eds.), pp. 441-474. Plant Resources of South - East Asia 5. Timber Trees: Minor commercial timbers. Backhuys Publishers, Leiden.

Lesica, P. & Allendorf, F.W. 1992. Are small populations of plants worth preserving? *Conservation Biology* **6**(1):135-139.

Lesica, P. & Martin, B. 2003. Effects of prescribed fire and season of burn on recruitment of the invasive exotic plant, *Potentilla recta*, in semiarid grassland. *Restoration Ecology* **11**(4):516-523.

Lindenmayer, D.B., Manning, A.D., Smith, P.L., Possingham, H.P., Fischer, I., Oliver, I. & McCarthy, M.A. 2002. The focal-species approach and landscape restoration: a critique. *Conservation Biology* **16**(2):338-351.

Linhart, Y.B. 1995. Restoration, revegetation and the importance of genetic and evolutionary perspectives, Proceedings of the Wildland Shrub and Arid Land Restoration Symposium, pp. 271–288. US Department of Agriculture.

Little, E.L. Jnr. & Wadsworth, F.H. 1964. Common Trees of Puerto Rico and the Virgin Islands. U.S Department of Agriculture Handbook 44, Washington D.C.

Liu, Q., Ge, S., Tang, H., Zhang, X., Zhu, G., & Lu, B-R. 2006. Phylogenetic relationships in *Elymus* (Poaceae:Triticeae) based on the nuclear ribosomal internal transcribed spacer and chloroplast trnL-F sequences. *New Phytologist* **170**:411-420.

Lockwood, J.L., Cassey, P. & Blackburn, T. 2005. The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution* **20**(5):223-228.

Loh J.P., Kiew, R., Kee, A., Gan, L.H. & Gan, Y-Y. 1999. Amplified fragment polymorphism (AFLP) provides molecular markers for the identification of *Caladium bicolor* cultivars. *Annals of Botany* **84**: 155-161.

Lonsdale, W.M. 1999. Global patterns of plant invasions and the concept of invisibility. *Ecology* **80**(5):1522-1536.

Loope, L.L. & Muller-Dombois, D. 1989. Characteristics of invaded islands, with special reference to Hawaii. In: J.A. Drake, H.A. Mooney, F. di Castri, R.H. (eds.) pp.257-280. Biological Invasions, a Global Perspective. Wiley, Chichester, U.K.

Lovegrove,, T.G., Zeiler, C.H., Greene, B.S., Green, B.W., Gaastra, R., & MacArthur, A.D. 2002. Alien plant and animal control and aspects of ecological restoration in a small "mailand island": Wenderholm Regional Park, New Zealand. In: Veitch, C.R. & Clout, M.N. (eds.) pp:155-163. Turning the Tide: the Eradication of Invasive Species. IUCN SSC Invasive Species Specialist group, IUCN, Gland, Switzerland and Cambridge, U.K.

Lucas, G. & Synge, H. 1978. The IUCN Plant Red Data Book. IUCN, Switzerland.

Lycos 2005. (available from http://members.lycos.co.uk/WoodyPlantEcology/ accessed 4th March 08).

Mabberley, D.J. 1997. The Plant Book. 2nd Edition. Cambridge University Press.

MacArthur, R.H. & Wilson, E.O. 1967. The Theory of Island Biogeography. Princeton University Press, Princeton, New Jersey.

Mace, G.M. & Lande, R. 1991. Assessing extinction threats: Towards a re-evaluation of the IUCN threatened categories. *Conservation Biology* **5**: 148-157.

Mack, R.N. 1996. Plant invasions: early and continuing expressions of global change. In: Huntely, B., Cramer, C., Morgan, A.V., Prentics, H.C. & Allen, J.R.M. (eds.) pp. 205-216. Past and Future Rapid Environmental Changes: Spatial and Evolutionary Responses of Terrestrial Biota. Springlag-Verlag, Berlin, Germany.

Mack, R.N., Simberloff, D.M., Lonsdale, W.M., Evan, H., Clout, M., Bazzaz, F.A. 2000. Biotic Invasions: causes, epidemiology, global consequences and control. *Ecological Applications* **10**(3):689-710.

MacKay, A.C., McGill, C.R., Fountain, D.W & Southward, R.C. Seed dormancy and germination of a panel of New Zealand plants suitable for re-vegetation. *New Zealand Journal of Botany* **40**(3):373-382.

Major, J. & Pyott, W.T. 1966. Buried viable seeds in two California bunchgrass sites and their bearing on the definition of a flora. *Vegetatio* **13**:253-282.

Manchester, S.J. & Bullock, J.M. 2000. The impacts of non-native species on UK biodiversity and the effectiveness of control. *Journal of Applied Ecology* **37**(5): 845-864.

Margules, C.R., Cresswell, I.D. & Nicholls, A.O. 1994. A scientific basis for establishing networks of protected areas. In: P.L. Forey, C.J. Humphries, & R.I. Vane-Wright (eds.), pp327-350. Systematics and Conservation Evaluation Oxford. Clarendon Press.

Martín, C., González-Benito, M-E., Iriondo, J.M. 1999. The use of genetic markers in the identification and characterization of three recently discovered populations of a threatened plant species. *Molecular Ecology* **8**(1):31-40.

Matthes, U., Gerrath, J.A. & Larson, D.W. 2003. Experimental restoration of disturbed cliff-edge forests in Bruce Peninsula National Park, Ontario, Canada. *Restoration Ecology* **11**(2):174-184.

Mauchamp, A. 1997. Threats from alien plant species in the Galápagos Islands. *Conservation Biology* **11**(1):260-263.

Mauchamp, A., Aldaz, I., Ortiz, E. & Valdebenito, H. 1998. Threatened species, a re-evaluation of the status of eight endemic plants of the Galápagos. *Biodiversity and Conservation* **7**: 97-107.

Maunder, M. 1992. Plant reintroduction an overview. Biodiversity & Conservation **1** 51:61.

Maunder, M., Culham, A. & Hankamer, C. 1998. Picking up the pieces; botanical conservation on degraded oceanic islands. In: Fiedler, P. L. & Karieva, P.M. (eds.), pp.317-344. Conservation Biology for the Coming Decade 2nd Edition. Chapman and Hall. International Thomson Publishing, Thomson Science.

Maunder, M., Culham, A., Alden, B., Zizka, G., Orliac, C. Lobin, W., Bordeu, A., Ramirez, J., Gliaamann-Gough, S. 2000. Conservation of the Toromiro tree: case study in the management of a plant extinct in the wild. *Conservation Biology* **14**(5):1341-1350.

Mayr, E. 1942. Systematics and the Origin of Species. Columbia University Press.

Mayr, E. 1954. Change of genetic environment and evolution. In: J.S. Huxley, A.C. Hardy and E.B. Ford (eds.), pp.156-180. Evolution as a Process. Allen & Unwin, London.

McCune, B. & Grace, J.B. 2002. Analysis of Ecological Communities. MJM Software design, Gleneden Beach, Oregan, USA.pp125-142

McCune, B. & Mefford, M.J. 1999. PC-ORD- Multivariate analysis of Ecological data Version 4.01. MjM Software, Gleneden Beach, Oregan. U.S.A.

McKenzie, D.P. 1972. Plate tectonics and sea-floor spreading. *American Scientist* **60**:425 - 435.

McMillan Browse, P. 1992. Plant Propagation. In: C. Brickell & K.A. Beckett (eds.). The Royal Horticultural Society's Encyclopaedia of Practical Gardening. Reed Books Limited, Michelin House, 81 Fulham Road, London SW3 6RB.

McMullen, C.K. 1987. Breeding systems of selected Galápagos Island angiosperms. *American Journal of Botany* **74**:1694-1705

Menges, E.S. 1991. The application of minimum viable population theory to plants. In: D.A. Falk & K.E. Holsinger (eds.), pp45-61. Genetics and Conservation of Rare Plants Oxford University Press.

Menges, E.S. & Gordon, D.R. 1996. Three levels of monitoring intensity for rare plant species. *Natural Areas Journal* **16**(3):227-237.

Menges, E.S. 1990. Population viability analysis for an endangered plant. *Conservation Biology* **4**:41-62.

Menges, E.S. 1992.Stochastic modelling of extinction in plant populations. In: Fiedler, P.L., Jain, S.K. (eds.), pp.253-276. Conservation Biology: The Theory and Practice of Nature Conservation, Preservation and Management. Chapman & Hall New York.

Merrill, E.D. & Perry, L.M. 1938. The Myrtaceae of China. *Journal of the Arnold Arboretum* **19**:191-247.

Merrill, E.D. & Perry, L.M. 1939. The myrtaceous genus *Syzygium* Gaertner in Borneo. *American Academy of Arts & Sciences* **18**:135-202.

Meudt, H.M & Clark, A.C. 2007. Almost forgotten or latest practice? AFLP applications, analyses and advances. *Trends in Plant Science* **12**:106-117.

Meyer, Jean-Yves. 2000. Preliminary review of the invasive plants in the Pacific islands (SPREP Member Countries). In: Sherley, G. (ed.), pp. 190. Invasive species in the Pacific: A Technical Review and Draft Regional Strategy. South Pacific Regional Environment Programme, Samoa. Middleton, B.A.

Middleton, B.A. 2003. Soil seed banks and the potential restoration of forested wetlands after farming. *Journal of Applied Ecology* **40**:1025-1034.

Millenium Ecosystem Assessment 2005. Ecosystems and Human-Wellbeing. Biodiversity Synthesis. World Resources Institute. Washington D.C.

Mookoto, P. Ching, L., & Nagai, G. 2002. Herbicidal Weed Control Methods for Pastures and Natural Areas of Hawaii. Cooperative Extension Service, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, Hawaii 96822. pp 1-33.

Moore, D.S & McCabe 2004. G.P. Introduction to the Practice of Statistics 4th Edition. W.H. Freeman and Company New York. pp.70.

Morton, J.F. 1987. Fruits of Warm Climates. Creative Resources Systems Inc. Box 890, Winterville, Nc, 28590.pp383-836.

Mueller-Dombois, D. & Fosberg, F.R. 1998. Vegetation of the Tropical Pacific Islands. Springer, New York.

Mueller-Dombois, D. & Loope, L.L. 1990. Some unique ecological aspects of oceanic island ecosystems. *Monographs in Systematic Botany from the Missouri Botanic Gardens* **32**:21-27.

Murphy, J. & Riley, H.P. 1962. A modified single solution method for the determination of phosphate in natural waters. *Acta Chimera* **27**:31-36.

Murphy, S. 2003. The origin and Evolutionary History of the Turlough form of *Ranunculus repens*. PhD. Thesis. University of Dublin, Trinity College.

Myers, J.H., Simberloff, D., Kuris, A.M. & Carey, J.R. 2000. Eradication revisited dealing with exotic species. *Trends in Ecology and Evolution* **15**(8):316-320.

Myers, J.H. & Bazely, D.R. 2003. Ecology and Control of Introduced Plants. Cambridge University Press.

Nagler, P.L., Hinojosa-Huerta, O., Glenn, E.P., Garcia-Hernandez, J., Romo, R., Curtis, C., Huete, A.R. & Nelson, S.G. 2005. Regeneration of native trees in the presence of invasive Saltcedar in the Colorado River Delta, Mexico. *Conservation Biology* **19**(6):1842-1852.

NCBI database (available from http://www.ncbi.nlm.nih.gov/Database/ accessed 4th March 08).

Nei, M. 1973. The theory and estimation of genetic distance. In: N.E. Morton (ed.), pp.45-54. Genetic Structure of Populations. University Hawaii Press, Honolulu.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583-590.

Nei, M. 1987. Molecular Evolutionary Genetics. New York. Columbia University Press.New York.

Nei-Li M., W. H. Lei 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings from the Natural Academy of Science.

Nielsen, L.R. 2004. Molecular differentiation within and among island populations of the endemic plant *Scalesia affinis* (Asteraceae) from the Galápagos Islands. *Heredity* **93**: 434-442.

O Brien, S.J. 1994. The cheetah's conservation controversy. *Conservation Biology* **8**: 1153-1155.

O'Dowd, D.J., Green, P.T. & Lake, P.S. 2003. Invasional "meltdown" on an oceanic island. *Ecology Letters* **6**:812-817.

Ogden, J. 1985. An introduction to plant demography with special reference to New Zealand trees. *New Zealand Journal of Botany* **23**:751-772.

Ogden, J.A.E. & Rejmánek, M. 2005. Recovery of native plant communities after control of a dominant invasive plant species, *Foeniculum vulgare*: Implications for management. *Biological Conservation* **125**:27-439.

Oldfield. S. 1999. Biodiversity: the UK Overseas Territories. D. Procter & L.V. Fleming, (eds.). Joint Nature Conservation Committee.

Olmstead, R.G. & Palmer, J.D. 1994. Chlorplast DNA systematics: a review of methods and data analysis. *American Journal of Botany* **81**:1205-1224.

Olesen, J.M. & Jordano, P. 2002. Geographical patterns in plant-pollinator mutualisitc networks. *Ecology* **83**:2416-2424

Oostermeijer, J.C.B., Luijten, S.H. & Den Nijs, J.C.M. 2003. Integrating demographic and genetic approaches in plant conservation. *Biological Conservation* **113**: 389-398.

Oostermeijer, J.G.B., Van Eijck, M.W., Van Leeuwen, N.C. & Den Nijs, J.C.M. 1995. Analysis of the relationship between allozyme heterozygosity and fitness in the rare *Gentiana pneumonanthe* L. *Journal of Evolutionary Biology* **8**: 739-757.

O'Rourke, P.J.1994. All the trouble in the world: the lighter side of famine, pestilence, destruction and death. London: Picador.

Otte, D. & Endler, J.A. 1989. Speciation and its consequences. Sinauer, Sunderland, Massachusetts. pp.679.

Ouborg, N.J., Piquot, Y., Van Groenendael, J.M. 1999. Population genetics, molecular markers and the study of dispersal in plants. *Journal of Ecology* **87**: 551-568.

Packard, S. and Mutel, C.F. 1997. The Tallgrass Restoration Handbook. Island Press, Washington, DC.

Page, W., Maunder, M. & Eastwood, A. 1995. A Conservation Assessment of Mauritian Taxa: Species Assessment Report. London. Conservation projects development Unit, Royal Botanic Gardens Kew.

Palacios, C. & Gonzalez-Candelas, F. 1997. Lack of genetic variability in the rare and endangered *Limonium cavanillescii* (Plumbaginaceae) using RAPD markers. *Molecular Ecology* **6**:671-675.

Palacios, C., Kresovich, S., González-Candelas, F.1999. A population genetic study of the endangered plant species *Limonium dufourii* (Plumbaginaceae) based on amplified fragment length polymorphism (AFLP). *Molecular Ecology* **8**:645-657.

Parker, P.G., Snow, A.A., Shug, M.D., Booton, G.C. & Fuerst, P.A. 1998. What molecules can tell us about populations: choosing and using a molecular marker. *Ecology* **79**: 361-382.

Parnell, 1999. Numerical analysis of Thai members of the *Eugenia-Syzygium* group (Myrtaceae). *Blumea* **44**:351-379.

Paulay, G. 1994. Biodiversity on Oceanic Islands: Its origin and extinction. *American Zoologist* **34**:134-144.

Peakall, R., Ebert, D., Scott, L.J., Meagher, P.F. & Offord, C.A. 2003. Comparative genetic study confirms exceptionally low genetic variation in the ancient and endangered relictual conifer, *Wollemia nobilis* (Araucariaceae). *Molecular Ecology* **12**: 2331-2343.

Pickett, S.T.A. & Parker, V.T. 1994. Avoiding the old pitfalls: opportunities in a new discipline. *Restoration Ecology* **2**:75-79.

Pimental, D., Zuniga, R., Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* **52**:273-288.

Prance, G.T. 1998. Beyond the Floras. *Australian Systemic Botany* **11**:153-159.

Preece, R.C. 1995. Systematic review of the land snails of the Pitcairn Islands. *Biological Journal of the Linnean Society* **56**: 273-207.

Primack, R.B. & Ros, J. 2002. Introduccion a la biologia de la conservacion. Ariel Ciencia, Barcelona.

Quammen, D. T1997. The Song of the Dodo: Island Biogeography in an Age of Extinctions. Pimlico.

Quinn, J.F. & Hastings, A. 1987. Extinction in subdivided habitats. *Conservation Biology* **1**:198-208.

Rabinowitz , D. 1981. Seven forms of Rarity. In : H. Synge (ed.), pp.205-217. The Biological Aspects of Rare Plant Conservation. Wiley, Chichester.

Rabinowitz, D., Cairns, S., & Dillon, T. 1986. Seven form of rarity and their frequency in the flora of the British Isles. In: M.E. Soule (ed.), pp182-204. Conservation Biology: The Science of Scarcity and Diversity. Sinauer, Sunderland, Massachusetts.

Randall, R.E. 1978. Theories and Techniques in Vegetation Analysis. pp.3. Oxford University Press, Oxford.

Rayment, M & White, A. 2007. Costing biodiversity in the U.K. Overseas Territories Royal Society for the Protection of Birds (RSPB) Report available from Residence 2, Royal William Yard, Plymouth, PL3 4JE.

Reed, D.H. 2005. Relationship between population size and fitness. *Conservation Biology* **19**(2):563-568.

Rejmánek, M., Pitcairn, M.J. & Bayer, D.E. 2000. Exotic Weeds: Current Situation Management Options and Priorities. A Californian Perspective. Auckland University Unpublished Manuscript.

Rejmánek, M. & & Richardson, D.M. 1996. What attributes make some plant species more invasive? *Ecology* 77(6):1655-1661.

Rhoades, C., Barnes, T. & Washburn, B. 2002. Prescribed fire and herbicide effects on soil processes during Barrens restoration. *Restoration Ecology* **10**(4):656-664.

Richards, A.J. 1997. Plant Breeding Systems. London. Chapman & Hall

Richarson, D.M. 1997. Forest trees as invasive aliens. *Conservation Biology* **12**(1):18-26

Richardson, D.M., P. Pysek, M. Rejmánek, M.G. Barbour, F.D. Panetta, and C.J. West. 2000. Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* **6**: 93-107

Ridout, C.J. & Donii, P. 1999. Use of AFLP in cereals research. *Trends in Plant Science* **4**:76-79.

Rippey, E., Rippey, J.J. & Dunlop, N. 2002. Management of indigenous and alien Malvaceae on islands near Perth, Western Australia. In: Veitch, C.R. & Clout, M.N. (eds.), pp254-259. Turning the Tide: the Eradication of Invasive Species. IUCN SSC Invasive Species Specialist group, IUCN, Gland, Switzerland and Cambridge, U.K.

Roberts, H.A. 1981. Seed banks in soils. *Advances in Applied Biology* **6**:1-55.

Robinson, G.R. & Quinn, J.F. 1988. Extinction, turnover and species diversity in an experimentally fragmented California annual grassland. *Oecologia* **76**:71-82.

Rønsted, N., Chase, M.W., Albach, D.C. & Bello, M.A. 2002. Phylogenetic relationships within *Plantago* (Plantaginaceae): evidence from nuclear ribosomal ITS and plastid trnL-F sequence data. *Biological Journal of the Linnean Society* **139**:323-338.

Ruiz-Jaen, M.C. & Aide, T.M. 2005. Restoration success: how is it being measured. *Restoration Ecology* **13**(3):569-577.

Russell, J.R., Hosein, F. Johnson, E. Waugh, R. & Powell, W.1993. Genetic differenciation of cocoa (*Theobroma cacao* L.) populations revealed by RAPD analysis. *Molecular Ecology* **2**:89-97.

Sanchez, P.A., Gichuru, M.P. & Katz, L.B. 1982. Organic matter in major soils of the tropical and temperate regions. In: 12th International; *Congress of Soil Science*, New Delhi, **1**:99-114.

Saunders, W.M.H. & Williams, E.G. 1955. Observations of the determination of Total Organic Phosphorus in soils. *Journal of Soil Science* **6** (2):255-267.

Scheffé, H. 1959. The Analysis of Variance. Wiley New York.

Schemske, D.W., Husband, B.C., Ruckelshaus, M.H., Goodwillie, C., Parker, I.M. & Bishop, J.G. 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* **75**(3): 584-606.

Schmid, R. 1972. A resolution of the *Eugenia-Syzygium* controversy (Myrtaceae). *American Journal of Botany* **59**:423-436.

Schneider, S., Rosessli, D., & Excoffier, L. 2000. ARLEQUIN Version 2.0. A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

SER 2002. Society for Ecological Restoration Science & Policy Working Group (2002). The SER Primer on Ecological Restoration (available from http://www.ser.org/ accessed 4th March 08).

SER 2004. Society for Ecological Restoration International Science and Policy working group. The SER International Primer on Ecological Restoration (available from http://www.ser.org/ accessed 4th March 08). Society for Ecological Restoration International, Tuscon, Arizona.

Shannon, C.E. & Weaver, W. 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana.

Sharma, R. & Verma, T. 2000. Effect of long-term addition of *Lantana* biomass on crop yields and n uptake in rice-wheat cropping in Himalayan acid Alfisols. *Tropical Agriculture* **77**:71-75.

Sharma, S.K., Knox, M.R. & Ellis, T.H.N. 1996. AFLP: Analysis of the diversity and Phylogeny of Lens and its comparison with RAPD analysis. *Theoretical and Applied Genetics* **93**:751-758.

Simberloff, D.M. 1995. Why do introduced species appear to devastate islands more than mainland areas? *Pacific Science* **49**:87-97.

Simberloff, D. & Stiling, P. 1996. Risks of species introduced for biological control. *Biological Conservation* **78**:185-192.

Simberloff, D.S. 1998. The contribution of population and community biology to conservation science. *Annual Review of Ecology and Systematics* **19**:473-511.

Simberloff, D. 2004. A rising tide of species and literature: a review of some recent books on biological invasions. *BioScience* **54**:247-254.

Smale, M.C. 2001. Ecological restoration of native forest at Aratiatia, North Island, New Zealand. *Restoration Ecology* **9**(1):28-37.

Smith, C.S., Lonsdale, W.M., & Fortune, 1999. When to ignore advice: invasion predictions and decision theory. *Biological Invasions***1**:89-96.

Smith, C.W. 1985. Impact of alien plants on Hawaii's native biota. In: C. P. Stone and M. J. Scott (eds.), pp186. Hawaii's Terrestrial Ecosystems: Preservation and Management. Co-Operative National Park Resources Studies Unit, University of Hawaii, Manoa.

Sokal, R.R. & Rolfe, F.J. 1995. Biometry 3rd Edition. W.H. Freeman and Co., New York.

Soltis, D.E., Soltis, P.S. & Doyle, J.J. 1998. Molecular Systematics of Plants II - DNA Sequencing. Kluwer Academic Publishers.

Soria, M.C., Gardener, M.R. & Tye, A. 2002. Eradication of potentially invasive plants with limited distributions in the Galapagos Islands. In: C. R. Veitch & M.N. Clout (eds.), pp.287-292. Turning the Tide: The Eradication of Invasive Species. IUCN SSC Invasive Species Specialist Group. IUCN, Gland, Switzerland and Cambridge, U.K.

Soulé, M.E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. In: M.E. Soulé & B.A. Wilcox (eds.), pp151-169. Conservation Biology - an Evolutionary and Ecological Perspective. Sinuaer Associates, Sunderland, Massachusetts.

Space, J.C. 2002. Pacific Island Ecosystems at Risk: Invasive Plants of the Pacific Islands. Version 3.3. (available from:http://www.hear.org/pier/ accessed 4th March 08). Institute of Pacific Island forestry, U.S. Forest Service, Honolulu.

Space, J.C. and Flynn, T. 2002. A Report to the Government of the Cook Islands on Invasive Species of Environmental Concern. USDA Forest Service, Honolulu. pp146.

Spencer, T. 1995. The Pitcairn Islands, South Pacific Ocean: plate tectonics and climatic conditions. *Biological Journal of the Linnean Society* **56**:13-42.

St. John, H. 1973. List and Summary of the Flowering Plants in the Hawaiian Islands. Pacific Tropical Botanic Garden.

St. John, H. 1987. An account of the flora of Pitcairn Island with new *Pandanus* species. In: *Pacific Plant Studies* **46** pp.65 Honolulu.

Stanturf, J.A., Schoenholtz, S.H., Schweitzer, C.J. & Shepard, J.P. 2001. Achieving restoration success: myths in bottomland hardwood forests. *Restoration Ecology* **9**:149-162.

Steadman, D.W. 1997. Human-caused extinctions of birds. In: M.L. Reaka-Kudla, W.E. Wilson & W.O. Wilson Biodiversity (eds.), pp139-161. Biodiversity II: Understanding and Protecting our Biological Resources. Joseph Henry Press, Washington, D.C.

Stevens, P.F. 1990. Nomenclature stability, taxonomic instrict, and flora writing-a recipe for disaster? In: P. Baas, K. Kalkman & R. Geesink (eds.), pp387-410. The Plant Diversity of Malesia. Kluwer Academic Publishers, Dordrecht, Netherlands.

Stevens, P.F. 1991. Character states, morphological variation and phylogenetic analysis - a review. *Systematic Botany* **16**(3):553-583.

Stewart, C.N. Jr. & Porter, D.M. 1995. The usefulness of RAPD profiling in biological conservation: an application to estimating the clonal variation in rare and endangered *Iliamna* in Virginia. *Biological Conservation* **74**:135-142.

Stout, J.C., Kells, A.R. & Goulson, D. 2002. Pollination of the invasive exotic shrub *Lupinus arboreus* (Fabaceae) by introduced bees in Tasmania. *Biological Conservation***106**:425-434.

Suding, K.N. 2005. The practice of restoration and the science of ecology. *Trends in Ecology & Evolution* **20**(11): 587-588.

Swart, J.A.A., Van der Windt, H.J. & Keulartz, J. 2001.Valuation of nature in conservation and restoration. *Restoration Ecology* **9**: 230-238.

Swofford, D.L., Olsen, G.J., Waddell, P.J. & Hillis, D.M. 1996. In: D.M. Hillis, C. Moritz & B.K. Mabpe (eds.), pp. 407-513. Molecular Systematics. Sinauer Associates, Sunderland, Massachussetts, USA.

Taberlet, P.L., Gielly, G., Pautou, G., & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105-1109.

Tear, T.H., Scott, J.M., Hayward, P.H. & Griffith, B. 1995. Recovery plans and the endangered species act: are criticisms supported by data? *Conservation Biology* **9**:182-195.

Teixeira, C.C. Fuch, F.D., Blotta, R.M. 1990. Effect of tea prepared from leaves of Syzygium jambos on glucose tolerance in non-diabetic subjects. *Diabetes Care* **13**(8):970-908.

Templeton, A.R. 1986. Co-adaptation and outbreeding depression. In: M.E. Soule (ed.), pp 91. Conservation Biology: The Science of Scarcity and Diversity. Sinauer, Sunderland, Massachusetts.

ter Braak, C.J.F. 1988 CANOCO - an extension of DECORANA to analyses species environment relationships. *Vegetatio* **75**:159-60.

Terbourgh, J. 1996. Keystone Plant Resources. In: M.E. Soule (ed.), pp.330-344. Conservation Biology: the Science and Scarcity of Diversity. Sunderland, Massachusetts: Sinauer.

Tero, N., Aspi, J., Siikamäki, P., Jäkäläniemi, A. & Tuomi, J. 2003. Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica*. *Molecular Ecology* **12**:2073-2085.

Towns D.R. and Ballantine W.J. 1993. Conservation and restoration of New Zealand island ecosystems. *Trends in Ecology and Evolution* **8**(12):453-457.

Townsend, C.R., Begon, M. & Harper, J.L. 2003. Essentials of Ecology 2nd Edition. Blackwell Publishing, Oxford.

Trujillo, E.E. 2005. History and success of plant pathogens for biological control of introduced weeds in Hawaii. *Biological Control* **33**:113-122.

Turner, C.E., Center, T.D., Burrows, D.W & Buckingham, G.R. 1998. Ecology an management of *Melaleuca quinquenervia*, an invader of wetlands in Florida, U.S.A. *Wetlands Ecology and Management* **5**:165-178.

Twyford, I.T. 1958. The Soil Resources of Pitcairn Island. Department of Agriculture Fiji.

Tye, A., Soria, M.C. & Gardener, M.R. 2002. A strategy for Galápagos weeds. In C.R. Veitch & M.N. Clout (eds.), pp. 336-341. Turning the Tide: the Eradication of Invasive Species. IUCN SSC Invasive Species Specialist Group. IUCN, Gland, Switzerland and Cambridge, U.K.

Underwood, A.J. 1997. Experiments in Ecology, their Logical Design and Interpretation using Analysis of Variance. pp.184. Cambridge University Press.

Van Andel, J. & Aroson, J. (ed.) 2005. Restoration Ecology: The New Frontier. Blackwell Publishing.

van der Valk, A.G. & Davis, C.B. 1978. The role of seed banks and the vegetation dynamics of prairie glacial marshes. *Ecology* **59**:322-335.

Van Ripper, C. III, Van Ripper, S.G., Goff, M.L. & Laird, M. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecological Monographs* **56**:327-344.

Vekemans, X. 2002. AFLP-SURV version 1.0. Distruibuted by the Author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgique.

Verardo, D.J., Froelich, P.N. & McIntyre, A. 1990. Determination of the organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 Analyser. *Deep Sea Research* **37** (1):157-165.

Vitousek, P.M. & Walker, L.R. 1989. Biological invasion by *Myrica faya* in. Hawai'i: plant demography, nitrogen fixation, ecosystem effects. *Ecological Monographs* **59**:247-265.

Vitousek, P.M., Walker, L.R., Whiteaker, L.D., Mueller-Dombois, D. & Matson, P.A. 1987. Biological invasion by *Myrica faya* alters ecosystem development in Hawaii. *Science* **238**:802-804.

Vitousek, P., D'Antonio, C., Loope, L. & Westbrooks, R. 1996. Biological invasions as global environmental change. *American Scientist* **84**:468-478.

Vitousek, P.M. 1988. Diversity and biological invasions on Oceanic Islands. In: E.O. Wilson (ed.), pp181-189. Biodiversity. Washington, National Academy Press. USA.

Vivrette, N.J. & Muller, C.H. 1977. Mechanism of invasion and dominance of coastal grasslands by *Mesembryanthemum crystallinum*. In: J. Van Andel & J. Aronson (eds.), pp320. 2005. The practice of restoration and the science of ecology. Blackwell Publishing.

Vos, P., Hogers, R., Bleeker, M., Rijans, M., Van de lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**(21):4407-4417.

Wade, M. 2005. Priorities for the control and management of alien invasive species on islands. *Biology and Environment*: Proceedings of The Royal Irish Academy **105B**(3):167-171.

Wagner, W.L., Herbst, D.R. & Sohmer, S.H. 1999. Manual of Flowering Plants of Hawaii. University of Hawaii Press, Honolulu.

Waldren, S. 2002. Conservation of Island Plant Populations and Communities (1.6.5.7). In: H. Heatwole (ed.) Encyclopaedia of Life Support Systems Volume 3: Oceanic Islands. UNESCO Publishing-Eolss Publishers, Paris, Oxford.

Waldren, S., Florence, J. & Chepstow-Lusty, A.J. 1995b. Rare and endemic vascular plants of the Pitcairn Islands: a conservation appraisal. *Biological Conservation* **74**:83-98.

Waldren, S., Florence, J., Chepstow Lusty, A.J. 1995a. A comparison of the vegetation communities from the islands of the Pitcairn group. *Biological Conservation* **56** (2):121-144.

Waldren, S., Kingston, N., Bingelli, P., Starmer, J. & Warren, J.1999b. Assessing the status of the Pitcairn Island flora - an integrated approach to conservation: Fifth International Botanic Gardens Congress.

Waldren, S., Weisler, M.I., Hather, J.G. & Morrow, D. 1999a. The non-native vascular plants of Henderson Island South-Central Pacific Ocean. *Atoll Research Bulletin* **463**:1-14.

Wallace, A.R. 1895. Island life 3rd Edition. Macmillan, London.

Watanabe, F.S. & Olsen, S.R. 1965. Test of an ascorbic acid method for determining P in water. *Soil Science* **85**:307-318.

Webb, C.J. & Kelly, D. 1993. The reproductive biology of the New Zealand flora. *Trends in Ecology and Evolution* **8**:442-447.

Wegener, A. 1915. The Origin of Continents and Oceans 4th Edition.New York, Dover Publishers pp.246.

Weisler, M.I. 1995. Henderson Island prehistory; colonisation and extinction on a remote Polynesian island. *Biological Journal of the Linnaean Society* **56**:377-404.

Weissing, K., Nyborn, H., Wolff, K. & Meyer, W. 1995. DNA Fingerprinting in Plants and Fungi. CRC Press, Boca, Ratan, N.J.

Welsh, J. & McClelland, M. 1990. Fingerprinting genomes using PCR with arbitary primers. *Nucleic Acids Research* **18**:7213-7218.

Welsh, S. L. 1998. Flora Societensis: A Summary Revision of the Flowering Plants of the Society Islands. E.P.S. Inc., Orem, Utah. pp. 420.

Westerbergh, A. & Saura, A. 1994. Genetic differentiation in endemic *Silene* (Caryophyllaceae) on Hawaiian Islands. *American Journal of Botany* **81**:1487-1493.

Whistler, W.A. 1991. Polynesian plant introductions. In: P.A. Cox & S.A. Banack (eds.), pp Islands, Plants and Polynesians: an Introduction to Polynesian Ethnobotany Dioscorides Press, Portland, Oregan.

Whittaker, R.J. & Fernandez-Palacios, J.M. 2007. Island Biogeography: Ecology, Evolution and Conservation. Oxford University Press

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., & Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**:6531-6535.

Williams, M.C. & Wardle, G.M. The invasion of two native Eucalypt forests by *Pinus radiata* in the Blue mountains, New South Wales, Australia. *Biological Conservation* **125**:55-64.

Williamson, M. & Fitter, A. 1996. The varying success of invaders. Ecology 77(6):1661-1666.

Williamson, M.H. 1988. Relationship of species number to area, distance and other variables. In: A.A. Myers and P.S. Giller (eds.), pp.91-115. Analytical Biogeography, an Integrated Approach to the Study of Animal and Plant Distributions. Chapman & Hall, London.

Wilson, E.O. 1992. Diversity of Life p. 340. Harvard University Press, Cambridge, MA

Wittzell, H. 1999. Chloroplast DNA variation and reticulate evolution in sexual and apomictic sections of dandlelions. *Molecular Ecology* **8**: 2023-2035.

Wolfe, K.H., Morden, C.W. & Palmer, J.D. 1992. Function and evolution of a minimal plastid genome from a nonphotosynthetic parasitic plant. *Proceedings of the National Academy of Sciences* U.S.A. **89**:10648-10652.

Wright, S. 1931. Evolution in Mendelian populations. *Genetics* **16**:97-159.

Wright, S. 1946. Isolation by distance under diverse systems of mating. *Genetics* **31**:39-59.

Wright, S.D. & Lees, A.M. 1996. Biodiversity conservation in the Island Pacific. In: A. Keast & S.E. Miller (eds.), pp. 445-461. The Origin and Evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: Patterns and Processes. Amsterdam, The Netherlands: SPB Academic Publishing.

Yeh, F.C., Boyle, T., Ye, Z. & Xiyan, J.M. 1999. POPGENE Version 1.21: Microsoft Windows-based freeware for population genetic analysis. University of Alberta and Centre for International Forestry Research.

Yelenik, S.G., Stock, W.D. & Richardson, D.M. 2004. Ecosystem level impacts of invasive *Acacia saligna* in the South African Fynbos. *Restoration Ecology* **12**(1): 44-51.

Young, T.P. 2000. Restoration ecology and conservation biology. *Biological Conservation* **92**:73–83.

Young, T.P., Petersen, D.A. & Clary, J.J. 2005. The ecology of restoration: historical links, emerging issues and unexplored realms. *Ecology Letters* **8**:662-673.

Zabeau, M & Vos, P. 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application, publication no. EP 0534858-A1, No. 92402629.7.

Zamith, L.R. Scarano, F.R. 2006. Restoration of a restinga sandy coastal plain in Brazil: survival and growth of planted woody species. *Restoration Ecology* **14** (1):87–94.

Zhivotovsky, L.A. 1999. Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology* **8**: 907-913.