Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Reproduction in selected New Zealand native ferns and their suitability for revegetation

A thesis presented in partial fulfilment of the requirements for the degree of

Masters of Science in Plant Biology

at Massey University Palmerston North, New Zealand

Matthew Denton-Giles

2006

ABSTRACT

The potential to use New Zealand native ferns for revegetation was assessed in laboratory, nursery and field experiments. Laboratory experiments indicated that the three native fern species, *Blechnum novae-zelandiae*, *Cyathea medullaris* and *Dicksonia squarossa*, had different maximum levels of spore germination. These differences also varied in response to seasonal changes in the environment. The effect of three soil conditioners on the germination of the same three species was minimal. Gametophytes appeared to be tolerant of low levels of maceration, as they were able to continue to grow and develop normally. Additional laboratory experiments indicated that *B. novae-zelandiae* employs a mixed mating system, which utilizes an "antheridiogen" signal.

The development of fern spores, laboratory propagated gametophytes and segmented rhizomes, was assessed in the nursery. Each experiment was applied with a hydroseeding mix of paper fibre, tackifier, fertilizer and water. Spore of *B. novae-zelandiae*, *C. medullaris* and *D. squarossa* failed to produce any long-lived gametophytes. The survival of laboratory propagated gametophytes of *B. novae-zelandiae*, *B. discolor* and *B. colensoi* was low. However, a large proportion of surviving *B. novae-zelandiae* gametophytes produced sporophytes. *B. novae-zelandiae* rhizome segments produced healthy young ferns within 3 months of application.

Field experiments were conducted on a sandstone/loess bank, 5 km east of Palmerston North. Aspects of the substrate were analysed including, pH, N, P and organic matter. The results indicated that the bank had a high soil pH, was deficient in several macronutrients and had no organic matter. Hydroseeding was applied using spore of the species *B. novae-zelandiae*, *C. medullaris* and *D. squarossa*. Hydroseeded spore failed to produce any visible gametophytes. Rhizome experiments using *B. novae-zelandiae* and *Microsorum pustulatum* were also established. Low water availability resulted in poor rhizome establishment.

The results suggest that there is great potential for utilizing native ferns in revegetation. Blechnum novae-zelandiae is the best species for revegetation in accordance to the results. Propagation via rhizome segmentation and gametophyte hydroseeding appear to be the most successful methods for establishing native ferns.

This TIF project was carried out in conjunction with Rural Supply Technologies, Manaaki Whenua Landcare Research, Massey University and FoRST New Zealand.

ACKNOWLEDGEMENTS

I would like to thank all the following people and organizations for their help, support and encouragement over the past two years:

An enormous thanks to my supervisor, John Clemens, whose encouragement and guidance kept me focused on the job at hand. I especially thank him for making time to meet with me at regular intervals, to chat about my ferns and their many wonders. A big thank you must also go to Craig Ross who remained "on call" throughout the project. His helpful suggestions and vast experience in the field contributed greatly to the success of this research.

A special thanks to Robert Coulson and Rural Supply Technologies Ltd, whose initiative and drive behind this project remained positive and enthusiastic. This project would not exist if it wasn't for your prowess. Thank you to, Anne Parkinson, Eddie Charlton, Phillip Yalden, Bruce McCaskie and Pat Reedy, who all had a role in this project, whether it was unblocking hydroseeder pumps, organising meetings, or supplying me with tools for my fern growing attempts.

I would like to acknowledge the New Zealand Foundation for Research Science and Technology (FoRST), for providing me with a Technology in Industry Fellowship. In addition, I would like to thank the providers of the J. P. Skipworth Scholarship, Coombs Memorial Bursary and Massey University Affinity Card Scholarship for their financial assistance.

On a more personal note I would like to thank my parents, Ken and Viv, for providing me with the means and encouragement to do what I enjoy most. I dedicate this work to you.

"Nature will forever endeavour".

TABLE OF CONTENTS

ABSTRACT	i
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	viii
LIST OF FIGURES	viii
1.0 INTRODUCTION	1
1.1 New Zealand native ferns	1
1.1.2 Distribution	1
1.2 The homosporous fern life cycle	3
1.2.1 Sexual mating systems and antheridiogen	5
1.2.2 Asexual reproduction	7
1.3 Spore germination and light	9
1.4 Fern propagation	12
1.4.1 In vitro propagation	12
1.5 Revegetation	14
1.5.1 Hydroseeding	15
1.5.2 Revegetation with native plants	18
1.6 Project aims	20
2.0 SPORE COLLECTION AND LABORATORY ANALYSES -	21
2.1 Overview	21
2.2 Materials and methods	22
2.2.1 Spore collection	22
2.2.2 Spore germination	24
2.2.2.1 Media preparation	24
2.2.2.2 Spore surface sterilization and sowing	24

	2.2.2.3 Assessing germination	25
	2.2.2.4 Fluorescein diacetate spore viability test	27
	2.2.3 Sexual mating systems	28
	2.2.3.1 Sporophyte production and gametophyte sexuality	28
	2.2.3.2 Antheridiogen	29
	2.2.4 Gametophyte transplantation and maceration	30
	2.2.4.1 Transplantation of gametophytes	30
	2.2.4.2 Maceration of gametophytes	31
	2.2.5 Estimating spore quantity	32
	2.2.6 Statistical analyses	32
2.3 R	2.3 Results	
	2.3.1 Spore germination	33
	2.3.1.1 Surface sterilization	33
	2.3.1.2 Spore germination in controlled, dark and natural conditions	34
	2.3.1.3 Seasonal variation in spore germination	38
	2.3.1.4 The effect of three polyacrylamides on spore germination	40
	2.3.1.5 Fluorescein diacetate spore viability test	41
	2.3.2 Sexual mating systems	43
	2.3.2.1 Sporophyte production and gametophyte sexuality	43
	2.3.2.2 Antheridiogen	45
	2.3.3 Gametophyte transplantation and maceration	49
	2.3.4 Estimating spore quantity	50
2.4 D	Discussion	51
	2.4.1 Spore germination	51
	2.4.2 Sexual mating systems	56
	2.4.3 Gametophyte transplantation and maceration	60
3.0	NURSERY EXPERIMENTS	61
3.1 C	Overview	61
3.2 N	Aaterials and Methods	62
	3.2.1 Nursery site preparation and experimental design	62
	3.2.1.1 Nursery site preparation	62

	3.2.1.2 Gametophyte propagation and establishment	63
	3.2.1.3 Rhizome establishment	66
	3.2.1.4 Spore establishment	66
3.3.2.	Statistical design and analyses	68
3.3 Results		70
3.3.1	Gametophyte experiments	70
	3.3.1.1 Gametophyte survival	70
	3.3.1.2 Sporophyte production on surviving gametophytes	72
3.3.2	Rhizome experiments	74
	3.3.2.1 Shoot emergence and growth	74
	3.3.2.2 The rhizome root system	76
3.3.3	Spore experiments	77
	3.3.3.1 Spore germination in the laboratory	77
	3.3.3.2 Gametophyte establishment in spore experiments	78
3.3.4	Adventive plant species in the nursery	80
3.4 Discuss	ion	82
3.4.1	Gametophyte experiments	82
3.4.2	Rhizome experiments	85
3.4.3	Spore experiments	87
4.0 FIEL	D EXPERIMENTS	91
4.1 Overvie	ew	91
4.2 Materia	als and Methods	92
4.2.1	Field experiment site preparation and analysis	92
	4.2.1.1 Site specifications	92
	4.2.1.2 Plot preparation	93
	4.2.1.3 Application	93
	4.2.1.4 Assessment	96
4.2.2	Statistical design and analyses	96
4.3 Results		97
4.3.1	Pre-experimental analyses	97
	4 3 1 1 Soil/substrate analysis	97

4.3.1.2 Spore germination in the laboratory	97
4.3.2 Hydroseeding fern spore in the field	99
4.3.2.1 Spore germination and gametophyte establishment	99
4.3.2.2 Vegetation cover	99
4.3.3 Fern rhizome experiment	101
4.3.3.1 Rhizome establishment	101
4.3.3.2 Rhizome viability	101
4.4 Discussion	103
4.4.1 Site characteristics	103
4.4.2 Hydroseeding native fern spore	105
4.4.3 Revegetation with rhizome segments	107
5.0 General Discussion	109
5.1 Overview	109
5.2 General Discussion and future work	110
5.2.1 The success of Blechnum novae-zelandiae	110
5.2.2 The development of commercial technology for native revegetation	112
5.3 Conclusions	115
References	117
Appendices	125
A DDC 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	4

LIST OF TABLES

Table 2.1	Blechnum novae-zelandiae gametophyte sexuality in the presence or	46
	absence of "antheridiogen".	
Table 2.2	Estimated quantity of dry spore in a 1 ml volume.	50
Table 3.1	Pre-application gametophyte propagation information.	63
Table 3.2	Gametophyte establishment and survival in nursery spore experiments.	78
Table 4.1	Characteristics of the field site substrate compared to pasture soil.	98

LIST OF FIGURES

Fig. 1.1	The homosporous fern life cycle of <i>Blechnum novae-zelandiae</i> .	3
Fig. 1.2	The phytochrome light-sensing system in fern spore.	11
Fig. 2.1	Manawatu spore collection site map.	22
Fig. 2.2	The effect of spore surface sterilization on spore germination.	33
Fig. 2.3	Photoblastic spore germination.	35
Fig. 2.4	Spore germination after a change in environment.	37
Fig. 2.5	Seasonal spore viability using germination data.	39
Fig. 2.6	The effect of various polyacrylamides on spore germination.	40
Fig. 2.7	Fluorescein diacetate and determining spore viability.	42
Fig. 2.8	In vitro propagation of ferns in the laboratory.	43
Fig. 2.9	Blechnum novae-zelandiae archegonia and a single antheridium.	43
Fig. 2.10	Intra-gametophytic selfing in B. novae-zelandiae.	44
Fig. 2.11	Blechnum novae-zelandiae mating systems observed in vitro.	44
Fig. 2.12	Dark spore germination in the presence/absence of "antheridiogen".	45
Fig. 2.13	Gametophyte development in the presence/absence of "antheridiogen".	46
Fig. 2.14	The effect of mature gametophytes on nearby developing spore.	48
Fig. 2.15	Gametophyte transplantation and maceration.	49
Fig. 2.16	Blechnum novae-zelandiae development after 66 days.	57
Fig. 3.1	Nursery layout including the irrigation system and data logger.	63
Fig. 3.2	The size distribution of B. novae-zelandiae, B. discolour and B. colensoi	65
	gametophytes used in the nursery gametophyte experiment.	

Fig. 3.3	Application of the rhizome experiment in the nursery	66
Fig. 3.4	A diagrammatical representation of a single ½ m² spore experiment plot.	67
Fig. 3.5	The Newbury shade-house map showing experiments and individual plots.	69
Fig. 3.6	Gametophyte survival in the nursery gametophyte experiments.	71
Fig. 3.7	Sequential development of sporophytes from gametophytes in the nursery.	72
Fig. 3.8	Mean number of gametophytes with sporophytes per replicate plot.	73
Fig. 3.9	The proportion of surviving gametophytes with sporophytes.	73
Fig. 3.10	Sequential development of <i>B. novae-zelandiae</i> shoots on chopped rhizome segments.	74
Fig. 3.11	Viability of rhizome segments over a period of 27 weeks.	75
Fig. 3.12	Rhizome shoot length over a period of 27 weeks.	75
Fig. 3.13	A diagrammatic representation of the position of roots, shoots and young	76
	fronds, on chopped rhizome segments of different orientations.	
Fig. 3.14	The extent of the root system in B. novae-zelandiae rhizome segments.	76
Fig. 3.15	Laboratory germination of spore used in nursery spore experiments.	77
Fig. 3.16	Gametophytes isolated from nursery spore experiments.	79
Fig. 3.17	Blechnum novae-zelandiae gametophytes showing increased necrosis at	79
	successive sampling dates.	
Fig. 3.18	Adventive plant species found in the nursery experiments.	80
Fig. 3.19	Adventive fern species and gametophytes found in nursery rhizome plots.	81
Fig. 3.20	A schematic representation of a B. novae-zelandiae rhizome.	86
Fig. 4.1	The field site, indicating loess and sandstone substrates.	92
Fig. 4.2	The application of fern spore using hydroseeding.	94
Fig. 4.3	The application of B. novae-zelandiae and Microsorum pustulatum	95
	rhizome segments.	
Fig. 4.4	The failure of the initial hydroseeding spore application.	95
Fig. 4.5	Laboratory germination of spore used in field spore experiments.	99
Fig. 4.6	Vegetation on a hydroseeded region compared with a non-hydroseeded	100
	region.	
Fig. 4.7	Vegetation cover on different treatment plots, over 6 months.	101
Fig. 4.8	Rhizome segment viability in the field, over 6 months.	102
Fig. 4.9	A rock seam in the field site.	104
Fig. 4.10	Changes in moss cover over the 6 month experimental period	105
Fig. 4.11	Evidence of erosion on the field site.	106
Fig. 4.12	A rhizome segment exhibiting frond browning and cell death in the field.	107