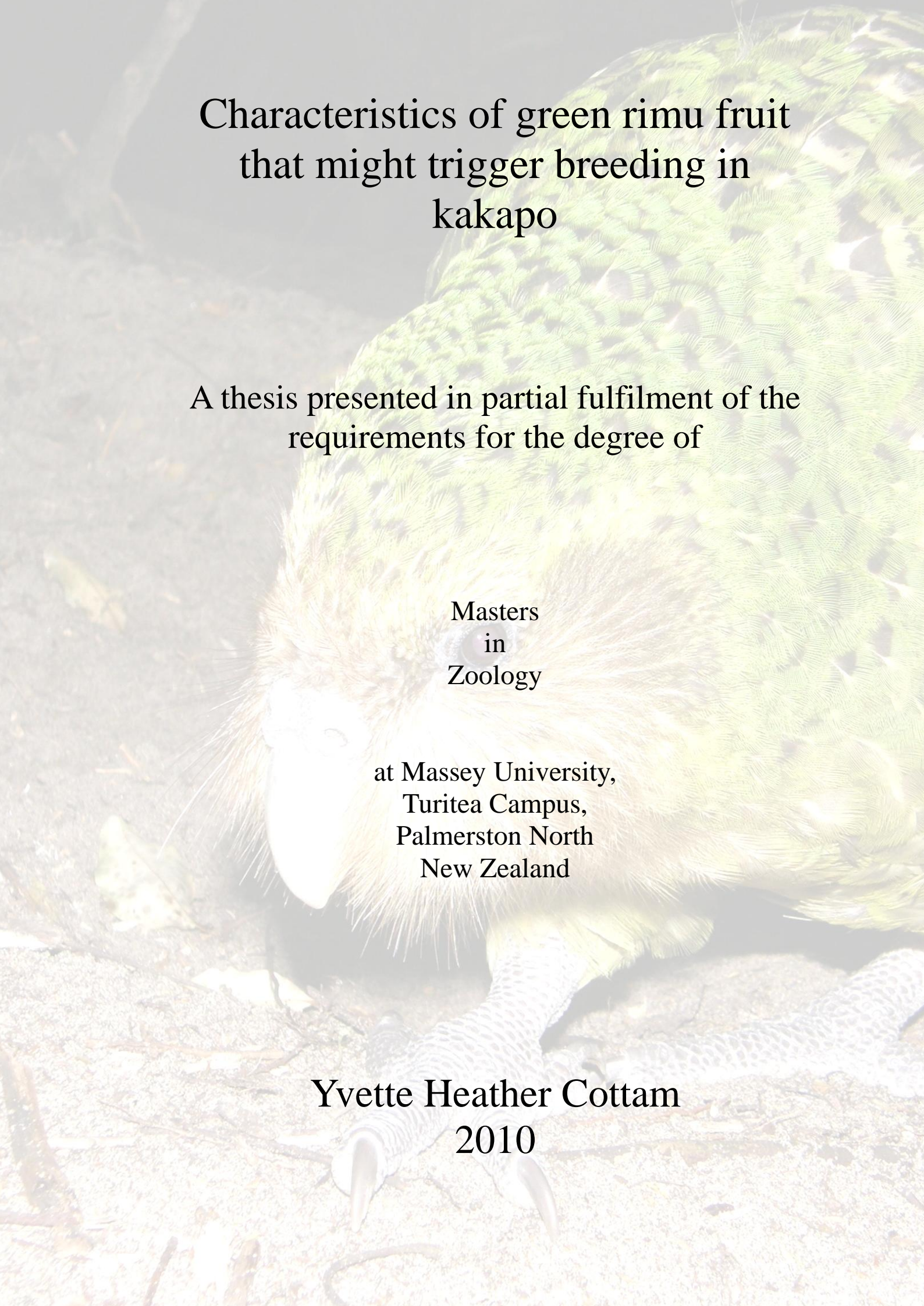


Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.



Characteristics of green rimu fruit
that might trigger breeding in
kakapo

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

Masters
in
Zoology

at Massey University,
Turitea Campus,
Palmerston North
New Zealand

Yvette Heather Cottam
2010

ABSTRACT

Species living in unpredictable habitats cannot prepare for a regular reproductive year, and must reproduce when conditions are favourable. The timing of reproduction of the seed predators involves anticipating an abundant food supply by tracking cues or triggers which initiate reproduction. These cues are poorly understood. The most supported theory is that a cue or cues is the size or abundance of the developing fruit crop, and possibly involves nutritional compounds in the crop.

The rimu (*Dacrydium cupressinum*) is a masting podocarp tree strongly linked to reproduction in kakapo (*Strigops habroptilus*). On Codfish Island kakapo only breed in rimu mast years and nesting is timed so chicks hatch when the rimu fruit crop (the sole food source for chicks) is due to ripen. This thesis investigates the theory that some component in unripe rimu fruit is involved in timing of nesting in kakapo. I characterised seasonal changes (over one year) in nutritional components (gross nutrients), polyphenolic compounds and other potential target polyphenolic substances with possible hormonal activity, including isoflavones and antioxidants, during rimu fruit development.

A number of the compounds found in rimu fruit are known to affect reproduction in other species and could be potential triggers for reproduction in kakapo. In non-mast years insufficient fruit is available to trigger reproduction and there is no breeding, supporting the idea that the abundance of the fruit crop is also involved in the timing of reproduction. The trigger for breeding in these birds could be ingestion of a threshold level of a trigger substance or substances, or, as in the antbird, it could be entirely visual. The most likely contenders for the trigger in green rimu fruit include increasing levels of crude protein, crude fat, individual fatty acids, dietary fibre (changing the digestibility of other nutrients), calcium, phosphorus, calcium, total polyphenolic content and antioxidant levels, some flavenoids or a combination of these.

It is possible that the physiological changes necessary for reproduction are triggered by the abundance of the unripe fruit crop but that nesting itself is triggered by changes in the green, undeveloped fruit associated with ripening.

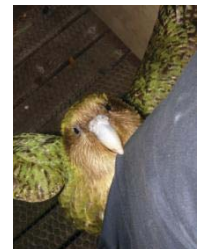
ACKNOWLEDGEMENTS

As with any field study this complex, numerous people were involved and deserve my sincere thanks. Thanks to all those people who helped with the tedious task of picking rimu fruit, a good thing to do at ‘Sealers Bay Resort and Spa’ on a rainy day.

Many thanks to all the kakapo team for some great times, and help with the whole project (in no particular order): Ness Smith, Tristan, Jo Leddington, Dana Boyte, Ursula Poole, Jeff Hall, Tristan Rawlence, Chris Birmingham, Daryl Eason, Diedre Vercoe, Becky Wilson, Sharon Trainor.

Many thanks to all involved in the laboratory analysis of the samples, Fliss Jackson and staff from the Nutrition lab (IFNHH) for the gross nutrient analyses, Karl Fraser and Geoff Lane from AgResearch for the HPLC/GC/MS analyses, Abdul Molan (IFNHH) for the polyphenolics and antioxidant analyses and Alasdair Noble (IFS) for some last minute stats advice.

Thanks to Sirocco, for many hours of entertainment and some wicked bruises!



Many thanks go to my supervisors, Ron Moorhouse, David Raubenheimer and Murray Potter for everything you have done for this project.

Special thanks to Ron for many things, including not laughing too hard the first time I shot a gun (luckily the moss was soft ☺), for making the entire study possible and for great last minute editing.



Thanks to David for some fun times doing fieldwork on Coddy, and great support during the final writing.

Thanks to Murray for all your help, including some great editing in the last few days of the marathon ☺

Thanks to Bruce, my partner, for putting up with me disappearing to Codfish for weeks at a time, and putting up with me when I was home trying to put this thesis together ☺.

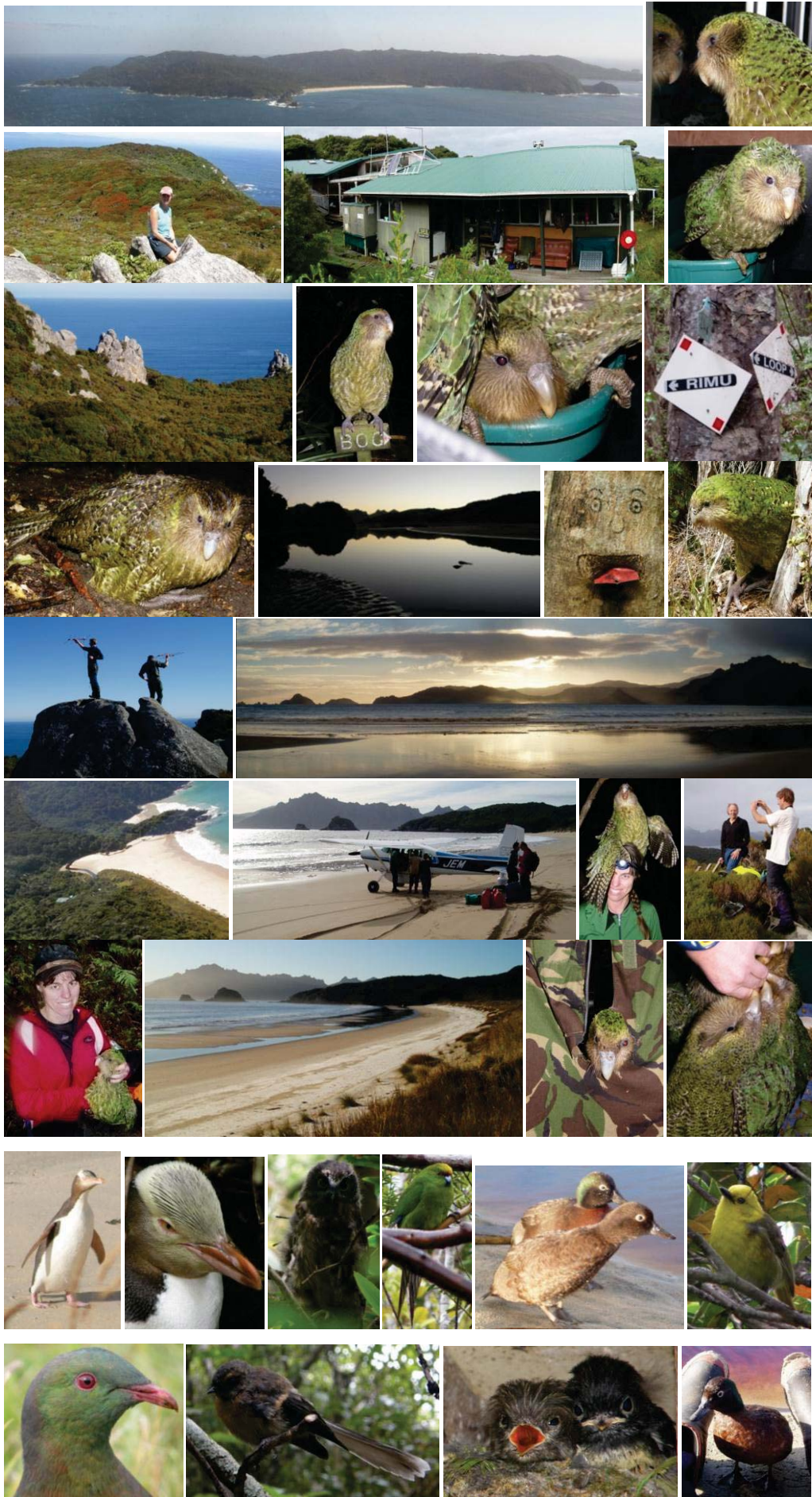




TABLE OF CONTENTS

ABSTRACT	i
ACKNOWLEDGEMENTS	ii
SECTION 1: COMPARATIVE OVERVIEW OF TRIGGERS TO BREEDING	1
1. Introduction	1
2. Bird Reproduction	2
3. Triggers.....	8
3.1. Masting:	9
3.2. Climatic Cues (temperature, rainfall):	12
3.3. Food Cues (abundance, nutritional, energetic):	14
3.4. Non-nutrient Chemical Cues	29
3.5. Visual Cues	32
4. Conclusions	32
SECTION 2: RESEARCH RESULTS.....	35
1. Introduction	35
2. Materials and methods.....	38
2.1. Study location	38
2.2. Sample collection.....	39
2.3 Chemical analyses	46
2.4. Statistical analyses	50
3. Results	50
3.1 Gross nutritional composition.....	50
3.2. Phenolic contents and antioxidant activity	61
3.3 Flavonoid identification	67
3.4 Oestrogenic Activity	75
4. Discussion.....	76
5. Summary and Conclusions	96
SECTION 3: BIBLIOGRAPHY	102

TABLE OF FIGURES

Figure 1. Codfish Island.....	39
Figure 2. Approximate location of each of the rimu trees from which fruit was collected, on Codfish Island.....	39
Table 1. Summary of samples collected over the year from individual, marked female rimu trees.....	41
Figure 3. An example of the amount of vegetation collected from one tree.....	42
Figure 4. Hoki sampling a branch of rimu fruit.	43
Figure 5. Rimu fruit on branches in May 2007 and February 2008.....	44
Figure 6. Rimu fruit collected from tree 1 over one year, with the least developed fruit in May 2007 to the most developed fruit in February 2008.....	44
Figure 7. Rimu fruit development from May 2007 to February 2008.	45
Figure 8. An example of the rimu fruit collected from the four aspects (branches facing north, east, south, west) around Tree 1, collected to examine within-tree variation.....	45
Table 2. Gross nutrient content of rimu fruit.	52
Figure 9. Dry matter, gross energy, crude protein, crude fat and ash in samples of fruit from six individual trees.....	53
Figure 10. Amount of crude protein and crude fat in rimu fruit over the year.	53
Figure 11. Total amount of gross nutrients (crude protein, crude fat, crude fibre, ash, sugars) in rimu fruit over the year.....	54
Figure 12. The amount of crude protein vs. crude fat and crude protein + crude lipid vs. calcium consumed per 100g of rimu fruit eaten.....	55
Table 3. Amino acid content of rimu fruit.	57
Table 4. Fatty acid content of rimu fruit.....	58
Figure 13. The ratio of linoleic (18:2):linolenic (18:3) fatty acids and the omega-3 and omega-6 fatty acid content of rimu fruit..	59
Figure 14. Levels of the four main fatty acids found in rimu fruit and the percent of each fatty acid as a proportion of the total fatty acids.....	60
Table 5. Correlation coefficients for relationships between assays.....	61
Table 6. Total polyphenolic content and antioxidant activities of rimu samples from seven composite samples and the aspect samples collected in February 2008.....	61
Figure 15. Average total polyphenolic content of the three dilutions of extracts of composite rimu fruit samples taken over one year.....	62

Figure 16. Total polyphenolic content of rimu fruit extract from six individual trees, four aspects per tree, collected in Feb 2008.	63
Figure 17. DPPH content of composite rimu fruit samples taken over one year.	64
Figure 18. DPPH content of rimu fruit from six individual trees, four aspects per tree, collected in Feb 2008.	64
Figure 19. FRAP content of composite rimu fruit samples taken over one year.	65
Figure 20. FRAP of rimu fruit from six individual trees, four aspects per tree, collected in Feb 2008.	65
Figure 21. CHA content of composite rimu fruit samples taken over one year.	66
Figure 22. CHA content of rimu fruit from six individual trees, four aspects per tree, collected in Feb 2008.	67
Figure 23. HPLC/GC/MS profiles of composite rimu fruit samples collected over one year.	69
Figure 24. HPLC/GC/MS profiles of rimu fruit from Tree 1 collected between November 2007 and February 2008.	70
Figure 25. HPLC/GC/MS profiles of rimu fruit from Tree 4 collected between November 2007 and February 2008.	71
Figure 26. HPLC/GC/MS profiles of rimu fruit from Tree 10 collected between November 2007 and February 2008.	72
Figure 27. Example of areas under the curve for the Feb-08 composite rimu fruit sample.	73
Figure 28. Areas under the curve expressed as a percentage of total area for each time point for composite rimu fruit samples taken over one year.	73
Figure 29. Areas under the curve expressed as a percentage of total area for each time point for three individual trees from Nov-07 to Feb-08.	74
Table 7. Summary of possible chemical triggers.	95