Insect bioactive capabilities of *Epichloë festucae var lolii* AR48 infected *Lolium perenne*

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ABSTRACT

As the modern world expands and develops, new innovative methodologies for more efficient and environmentally friendly agricultural practices are required. Loss of crops through abiotic (*e.g.* drought) and biotic (*e.g.* herbivory) stresses has a major effect on the success of an agricultural industry. For animal production pasture crops are a key aspect of animal husbandry and directly affects yield and health. Symbiotic fungi belonging to the genus *Epichloë* form associations with cool season forage grasses and have been exploited as a new innovative method for insect pest management. Ryegrass infected with the asexual *E. festucae* var *lolii* strain AR48 has insect bioactivity against both the stem boring fly (SBF-*Ceradontha australis*) and cutworm moth caterpillar (CC -*Agrotis ipsilion*). The bioactive/s targeting both insects is currently unknown. The aim of this thesis was to identify the gene/s and/or bioactive/s present in AR48 infected ryegrass that have bioactivity against the SBF and/or CC. Two approaches were taken; the known insect bioactive secondary metabolite pathways in *Epichloë* were investigated in AR48 through bioinformatics and mass spectrometry, and the gene 'makes caterpillars floppy' (*mcf*), encoding an insect toxin like protein, was investigated through reverse genetics and insect bioactivity trials.

A new indole diterpene compound (IDT) was identified in AR48 infected plant material and this compound was absent in other *Epichloë* strains that do not have SBF and CC bioactivity. The same *mcf* gene allele as that present in the *E. typhina mcf* model, previously identified as having CC bioactivity, is present and predicted to be functional in AR48. The other *Epichloë* strains also have *mcf* genes predicted to be functional, however the *mcf* allele is different to the bioactive *E. typhina mcf* model. Overall, this project was able to identify a new IDT compound with potential insect bioactivity as well as identify two *Epichloë mcf* gene alleles that potentially have differing insect bioactivities.

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TABLE OF CONTENTS

1.0 Introduction.....1

1.1 *Epichloë* endophyte as a commercialised bioprotectant.....3

1.1.1 Insect pests of *Lolium perenne*.....3

1.1.2 Commercialisation of *Epichloë* as an insect biocontrol agent.....5

1.1.3 Novel insect bioprotection of *Epichloë festucae* var *lolii* AR48 infected ryegrass.....7

1.2 *Epichloë* endophyte molecular characterisation.....10

1.2.1 *Epichloë* in culture and *in planta* morphologies.....10

1.2.2 *Epichloë* asexual and sexual life cycles.....11

1.2.3 Establishment and maintenance of *Epichloë*-host symbiosis.....13

1.2.4 Fungal secondary metabolite gene clusters.....14

1.2.5 Regulation of fungal secondary metabolites.....15

1.2.6 *Epichloë in planta* induced bioactive alkaloids.....17

1.2.6.1 *Epichloë* ergot alkaloids.....19

1.2.6.2 *Epichloë* 1-Aminopyrrolizidines.....19

1.2.6.3 *Epichloë* pyrrolopyrazines.....20

1.2.6.4 *Epichloë* indole-diterpenes.....21

1.3 *Penicillium* and *Epichloë* indole-diterpene characterisation.....21

1.3.1 *Penicillium* indole-diterpenes.....22

1.3.2 *Epichloë* indole-diterpenes.....24

1.4 Makes caterpillars floppy (Mcf) as a possible *Epichloë* insect bioactive….27

1.4.1 *Photorhabdus luminescens* Mcf.....27

1.4.2 *Pseudomonas fluorescens* FitD.....29

1.4.3 *Epichloë* Mcf.....29

1.5 AR48 infected *Lolium perrene* insect bioactivity.....30

1.5.1 Agromyzidae characterisation.....30

1.5.1.1 Agromyzidae life cycle.....31

1.5.1.2 Distribution of Agromyzidae New Zealand.....31

1.5.1.3 *Cerodontha australis* characterisation.....32

1.5.1.4 Economic impact and control management of Agromyzidae.....33

1.5.2 Noctuidae characterisation.....35

1.5.2.1 *Agrotis ipsilon characterisation*.....35

1.5.2.2 *Agrotis ipsilon* life cycle.....36

1.5.2.3 Economic impact and control management of *Agrotis ipsilon*.....37

1.6 Aims.....39

2.0 Materials and Methods.....41

2.1 Molecular and biological materials.....43

2.1.1 Details of strains used in this study.....43

2.1.2 Details of plasmids used in this study.....46

2.1.3 Details of primers used in this study.....47

2.2 Sterile conditions.....50

2.3 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1.....50

2.3.1 *Epichloë festucae* growth conditions.....50

2.3.2 *Penicillium paxilli* growth conditions.....50

2.3.3 *Epichloë festucae* genomic DNA extraction.....50

2.3.4 *Epichloë festucae* crude DNA extraction.....51

2.3.5 *Penicillium paxilli* crude DNA extraction.....52

2.3.6 *Epichloë festucae* glycerol stocks.....52

2.3.7 *Penicillium paxilli* glycerol stocks.....52
2.3.8 *Penicillium paxilli* protoplasting preparation.....52
2.3.9 *Penicillium paxilli* protoplast transformation.....53
2.3.10 *Penicillium paxilli* spore stocks.....54
2.3.11 Bioinformatics analysis of *Epichloë festucae* insect bioactive genes.....54
2.3.12 *Lolium perenne* seed accession.....54
2.3.13 *Lolium perenne* seed DNA extraction.....54
2.3.14 Simple sequence repeat (SSR) polymerase chain reaction (PCR) 55
2.3.15 SSR genotyping.....55
2.3.16 DNA sequencing.....55
2.3.17 Indole-diterpene extraction from *Epichloë festucae* herbage and seed.....56
2.3.18 Indole-diterpene extraction from *Penicillium paxilli* cultures.....56
2.3.19 Chromatography of indole-diterpene extracts for triple quadrupole (TSQ) analyses.....56
2.3.20 Chromatography of indole-diterpene extracts for triple quadrupole (TSQ) analyses.....57
2.3.21 Mass spectrometry analysis TSQ of indole-diterpenes from *Epichloë festucae*.....57
2.3.22 Mass spectrometry analysis LTQxl of indole-diterpenes from *Epichloë festucae*.....57
2.3.23 Mass spectrometry analysis LTQxl of indole-diterpenes from *Penicillium paxilli*.....58
2.3.24 Fragmentation in tree generation for *Epichloë festucae* indole-diterpenes.....59

2.4 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass.....59

2.4.1 Bioinformatics analysis of makes caterpillars floppy (mcf).....59

2.4.2 *Escherichia coli* growth conditions.....60
2.4.3 *Escherichia coli* plasmid extraction.....60
2.4.4 Chemically competent *Escherichia coli* DH5α cells.....60
2.4.5 *Escherichia coli* DH5α cell transformation.....60
2.4.6 *Escherichia coli* glycerol stocks.....61
2.4.7 *Escherichia coli* plasmid design.....61
2.4.8 *Epichloë festucae* protoplast preparation.....61
2.4.9 *Epichloë festucae* protoplast transformation.....62
2.4.10 *Epichloë festucae* spore isolation.....62
2.4.11 *Lolium perenne* growth conditions.....63
2.4.12 *Lolium perenne* seed sterilisation.....63
2.4.13 *Lolium perenne* seedling inoculation.....63
2.4.14 *Epichloë festucae* infected *Lolium perenne* plant immunoblotting.....64
2.4.15 *Epichloë festucae* infected *Lolium perenne* plant macroscopy....64
2.4.16 *Epichloë festucae* infected *Lolium perenne* plant microscopy.....64
2.4.17 DNA gel extraction.....65
2.4.18 DNA column purification.....65
2.4.19 DNA Qubit.....65
2.4.20 DNA Nanophotometer.....65
2.4.21 PCR.....66
2.4.22 DNA agarose gel electrophoresis.....66
2.4.23 Gibson assembly.....66
2.4.24 Clone checker.....66
2.4.25 Restriction analysis.....67
2.4.26 *Epichloë festucae* Fg1 *mcf* whole gene replacement construct...67
2.4.27 *Epichloë festucae* Fg1 *mcf* 5' gene replacement construct.....67
2.4.28 *Epichloë festucae* var *lolii* AR48 *mcf* gene complementation construct.....68
2.4.29 *Epichloë festucae* var *lolii* AR48 *idtP* gene complementation construct.....68

2.4.30 *Epichloë festucae* var *lolii* AR48 *idtQ* gene complementation construct.....68
2.4.31 DIG probe preparation for Southern blotting.....68
2.4.32 Genomic DNA preparation for Southern blotting.....69
2.4.33 Gel electrophoresis for Southern blotting.....69
2.4.34 DNA blotting for Southern blotting.....70
2.4.35 Hybridization of the probe for Southern blotting.....70
2.4.36 Stringency washes for Southern blotting.....71
2.4.37 Immunological detection for Southern blotting.....71

2.5 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass.....71
2.5.1 Stem boring fly whole plant choice trial plant preparation.....71
2.5.2 Stem boring fly preparation.....72
2.5.3 Stem boring fly whole plant choice trial.....72
2.5.4 Cutworm moth caterpillar whole plant choice trial plant preparation.....73
2.5.5 Cutworm moth caterpillar preparation.....73
2.5.6 Cutworm moth caterpillar whole plant choice trial.....73
2.5.7 Cutworm moth or porina caterpillar detached tiller no choice trial plant preparation.....74
2.5.8 Porina caterpillar preparation.....74
2.5.9 Cutworm moth or porina caterpillar detached tiller no choice trial.....74
2.5.10 Light brown apple moth caterpillar artificial diet no choice trial plant preparation.....75
2.5.11 Light brown apple moth caterpillar preparation.....75
2.5.12 Light brown apple moth caterpillar artificial diet no choice trial.....75

3.0 Results.....77

3.1 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1.....79

3.1.1 Analysing the functionality and presence of the bioactive secondary metabolite genes in the genome of AR48 and Fg1 through bioinformatics.....79

3.1.2 Detection of the bioactive secondary metabolite compounds in AR48 and Fg1 infected ryegrass through mass spectrometry.....88

3.1.3 Analysing the structure of any newly identified compounds through mass spectrometry.....89

3.1.4 New indole-diterpene compound three synthesis through *Penicillium paxilli* complementation.....90

3.2 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass.....99

3.2.1 Analysing the distribution and functionality of the *mcf* gene within the *Epichloë festucae* species through bioinformatics.....99

3.2.2 Analysing the distribution and functionality of the *mcf* gene within the *Epichloë* genus through bioinformatics.....100

3.2.3 Analysing the location of the *mcf* gene within the *Epichloë* genus through bioinformatics.....105

3.2.4 Analysing the distribution, functionality, and location of the *mcf* gene outside the *Epichloë* genus through bioinformatics.....108

3.2.5 Analysing domain composition of Mcf/FitD proteins through bioinformatics.....112

3.2.6 Analysing the potential bioactivitiy of Mcf through reverse genetics of *Epichloë festucae* Fg1.....117

3.2.7 Analysing the potential bioactivitiy of Mcf through reverse genetics in *Epichloë festucae* var *lolii* AR1 *and Epichloë festucae* var *lolii* AR37.....119

3.3 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass.....125

3.3.1 Design and perform a stem boring fly (Cerodontha australis) whole plant choice bioactivity trial on a range of wild type *Epichloë*-infected ryegrass.....125

3.3.2 Design and perform a cutworm moth caterpillar (*Agrotis ipsilon*) whole plant choice bioactivity trial on a range of wild type *Epichloë*-infected ryegrass.....129

3.3.3 Design and perform a cutworm moth caterpillar (*Agrotis ipsilon*) detached tiller no choice bioactivity trial on a range of wild type and *mcf* deletion *Epichloë*-infected ryegrass.....132

3.3.4 Design and perform a a porina caterpillar (*Wiseana* spp) detached tiller no choice bioactivity trial on a range of wild type *Epichloë*-infected ryegrass.....137

3.3.5 4 Design and perform a light brown apple moth caterpillar (*Epiphyas postvittana*) bioactivity trial on a range of wild type *Epichloë*-infected ryegrass.....140

4.0 Discussion.....145

4.1 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1.....147

4.1.1 Fg1 as a model AR48 strain.....147

4.1.2 AR48 and Fg1 *in planta* insect bioactivity potential predicted through bioinformatics.....148

4.1.3 AR48 and Fg1 new indole-diterpene compound three structure predicted through mass spectrometry.....150

4.1.4 AR48 and Fg1 new indole-diterpene compound three synthesis predicted through bioinformatics.....152

4.2 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass.....157

4.2.1 Mcf gene characterisation in *Epichloë*.....157

4.2.2 Proposed role of *Epichloë* Mcf.....159

4.2.3 *Epichloë festucae* Fg1 Δmcf characterisation.....161

4.3 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass.....162

4.3.1 AR48 and Fg1 new indole-diterpene potential bioactivity.....162

4.3.2 *Epichloë* Mcf infected ryegrass potential bioactivity.....163

4.3.3 Difference in AR48 and *Epichloë festucae* var *lolii* AR47 infected ryegrass bioactivities.....166

4.3.4 Potential alternative bioactives for AR48 infected ryegrass novel insect bioactivity.....167

4.3.5 Comparison between different insect bioactivity trial methodologies.....168

5.0 Conclusion.....175

5.1 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae var lolii* AR48 and *Epichloë festucae* Fg1.....177

5.2 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected Ryegrass.....178

5.3 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass.....178

5.4 Overall conclusion of the project.....179

6.0 Future experiments.....181

6.1 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae var lolii* AR48 and *Epichloë festucae* Fg1.....183

6.1.1 AR48 and Fg1 genome assembly.....183

6.1.2 AR48 and Fg1 new indole-diterpene compound three ` structure.....183

6.1.3 AR48 and Fg1 new indole-diterpene compound three synthesis pathway.....184

6.1.4 AR48 and Fg1 new indole-diterpene compound three phylogenetic distribution.....184

6.2 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass.....185

6.2.1 *Epichloë* mcf allelic variants.....185

6.2.2 *Epichloë* Mcf domain functions and bioactivity mechanism.....185

6.2.3 *Epichloë* Mcf in culture, in planta, and target insect localisation.....186

6.3 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass.....186

6.3.1 Epichloë festucae AR48 and Fg1 new indole-diterpene compound three insect bioactivity.....186

6.3.2 *Epichloë festucae* Fg1 mcf reverse genetics.....187

7.0 Limitations.....189

7.1 Analyse the bioactive secondary metabolite pathways in *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1.....191

7.1.1 AR48 and Fg1 new indole diterpene three unresolved structure.....191
7.1.2 AR48 and Fg1 new indole diterpene three unresolved synthesis pathway.....191

7.2 Investigate "makes caterpillars floppy" (Mcf) as a potential bioactive for the new bioactivities observed in *Epichloë festucae* var *lolii* AR48 infected ryegrass.....192
7.2.1 *Epichloë* limited genetic manipulation capabilities.....192
7.2.2 *Epichloë* in planta only expressed secondary metabolites.....193

7.3 Test the insect bioactivity capabilities of *Epichloë festucae* var *lolii* AR48 and *Epichloë festucae* Fg1 infected ryegrass.....193
7.3.1 *Epichloë festucae* var *lolii* complex interaction between bioactive secondary metabolite profiles and insect bioactive capabilities.....193
7.3.2 Non-model system insect species.....194

8.0 Appendices.....195

9.0 References.....279

LIST OF FIGURES

Figure 1.1: Stem boring fly (SBF-*Cerodontha australis*) bioactivity observed in an Argentine stem weevil (ASW- *Listronotus bonariensis*) plot trial.....8

Figure 1.2: The detection of cutworm moth caterpillar (CC-*Agrotis ipsilon*) bioactivity in a range of perennial ryegrass (top label) and endophyte (bottom label) combinations under laboratory conditions 2008.....9

Figure 1.3: The detection of cutworm moth caterpillar (*Agrotis ipsilon*) bioactivity in a range of perennial ryegrass and endophyte combinations under laboratory conditions 2012.....10

Figure 1.4: Fungal intercalary growth.....11

Figure 1.5: Diagram of both asexual and sexual life cycles of *Epichloë* species.....13

Figure 1.6: Simplified biochemical pathways that have been proposed for the production of secondary metabolites produced by *Epichloë* species....18

Figure 1.7: Ergot alkaloid (EAS) gene cluster in *Epichloë festucae* E2368.....19

Figure 1.8: Loline (LOL) gene cluster in *Epichloë festucae* E2368.....20

Figure 1.9: The structure of peramine (A) and the corresponding *perA* gene in *Epichloë festucae* Fl1 (B).....21

Figure 1.10: Indole diterpene (IDT/LTM) gene cluster in *Epichloë festucae* Fl1.....21

Figure 1.11: Simplified proposed paxilline biosynthetic pathway in *Penicillium paxilli*.....23

Figure 1.12: Indole diterpene (IDT/LTM) gene cluster in *Epichloë festucae* Fl1 strain.....25

Figure 1.13: Proposed lolitrem B biosynthetic pathway in *Epichloë festucae* Fl1 strain.....26

Figure 1.14: Comparison of makes caterpillars floppy (mcf)-like proteins.....27

Figure 1.15: Schematic diagram of the morphology of Diptera.....34

Figure 1.16: *Ceradontha australis* (stem boring fly-SBF) 34

Figure 1.17: *Agrotis ipsilon* (cutworm moth (CM) and cutworm moth caterpillar (CC)) 37

Figure 3.1: Organisation of indole-diterpene (IDT) genes in different *Epichloë festucae* strains.....81
Figure 3.2: Indole-diterpene (IDT) *IdtF* and *idtK* gene alignment in different *Epichloë festucae* strains.....82
Figure 3.3: *PerA* gene alignment for different *Epichloë festucae* strains.....83
Figure 3.4: Simple sequence repeat (SSR) dendrogram of *Epichloë festucae* strains using 23 loci.....84
Figure 3.5: Amino acid sequence alignment of indole-diterpene (IDT) IdtP in *Epichloë festucae* strains AR48, Fg1, Fl1, E2368, AR1, AR37, and AR5 using Geneious.....86
Figure 3.6: Amino acid sequence alignment of indole-diterpene (IDT) IdtQ in *Epichloë festucae* strains AR48, Fg1, Fl1, E2368, AR1, AR37, and AR5 using Geneious.....87
Figure 3.7: Liquid chromatography mass spectrometry (LC-MS) analysis of indole-diterpenes (IDTs) from *Epichloë festucae* infected ryegrass.....92
Figure 3.8: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of paxitriol from *Epichloë festucae* Fl1 infected ryegrass.....93
Figure 3.9: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of terpendole E from *Epichloë festucae* Fl1 infected ryegrass.....94
Figure 3.10: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of new indole-diterpene compound three from *Epichloë festucae* AR48 and Fg1 infected ryegrass.....95
Figure 3.11: Simplified lolitrem B pathway in *Epichloë.* Genes (single letter *e.g.* indole-diterpene (IDT) = *idtP* = P) next to associated reaction.....98
Figure 3.12: Analysis of the predicted makes caterpillars floppy (*mcf*) gene structure in a range of *Epichloë festucae* strains.....102
Figure 3.13: Analysis of the predicted makes caterpillars floppy (*mcf*) gene structure in a range of *Epichloë* species.....104
Figure 3.14: Identification of genes surrounding makes caterpillars floppy (*mcf*) within the *Epichloë festucae* Fl1 genome of chromosome 7.....106
Figure 3.15: Analysis of makes caterpillars floppy (*mcf*) syntony in *Epichloë...*.....107

Figure 3.16: Identification of other fungal makes caterpillars floppy (*mcf*) genes outside *Epichloë*.....110
Figure 3.17: Domain analysis of makes caterpillars floppy (Mcf) and P. fluoresces insect toxin (FitD) proteins using online tools (Pfam, InterPro Scan, Phyre2 or Hhpred) in a range of species.....116
Figure 3.18: Strategy for deletion of the whole *Epichloë festucae* Fg1 makes caterpillars floppy (*mcf*) gene.....121
Figure 3.19: Strategy for deletion of the 5' end of the *Epichloë festucae* Fg1 makes caterpillars floppy (*mcf)* gene.....122
Figure 3.20: NBT/BCIP strained Southern blot of *Bam*H1 genomic digest (1 µg) of *Epichloë festucae* Fg1 wild type (WT) and makes caterpillars floppy (*mcf*) gene deletion (Δ*mcf*)(#117) strains probed with digoxigenin (DIG)-11-dUTP linear pTM05 insert probe.....123
Figure 3.21: Culture phenotype of *Epichloë festucae* Fg1 wild type (WT) and makes caterpillars floppy (*mcf*) gene deletion (Δ*mcf*) strains.....124
Figure 3.22 Stem boring fly (SBF-*Cerodontha australis*) whole plant choice trial (SWPC) set up.....127
Figure 3.23: Stem boring fly (SBF-*Cerodontha australis*) whole plant choice trial (SWPC) results.....128
Figure 3.24: Cutworm moth caterpillar (CC-*Agrotis ipsilon*) whole plant choice trial (CWPC) set up.....130
Figure 3.25: Cutworm moth caterpillar (CC-*Agrotis ipsilon*) whole plant choice trial (CWPC) results.....131
Figure 3.26: Cutworm moth caterpillar (CC-*Agrotis ipsilon*) detached tiller no choice trial (CDTN) set up.....134
Figure 3.27: Cutworm moth caterpillar (CC-*Agrotis ipsilon*) detached tiller no choice trial (CDTN) survival rate (%) results.....135
Figure 3.28: Cutworm moth caterpillar (CC-*Agrotis ipsilon*) detached tiller no choice trial (CDTN) daily weight change results.....136
Figure 3.29: Porina caterpillar (PC-*Wiseana* spp) detached tiller no choice trial (PDTN) results.....139

Figure 3.30: Light brown apple moth caterpillar (LBAM-*Epiphyas postvittana*) artificial diet no choice trial (LADN) results.....143
Figure 4.1: Schematic of the diversity of early pathway indole diterpene (IDT) compounds produced by the four core genes (G, C, M, and B- coloured) in fungi.....155
Figure 4.2: Simplified lolitrem B pathway in *Epichloë*.....156
Figure 4.3: Graphical representation of the genomic location of secondary metabolite genes that produce bioactive compounds, in *Epichloë festucae* Fl1 complete assembled genome.....170
Figure 4.4: Proposed mode of action for the *Epichloë* makes caterpillars floppy (Mcf) protein based on the *Clostridium difficile* TcdA and TcdB toxins.....171

LIST OF TABLES

Table 1.1: Comparison of economically important insect pests of ryegrass in New Zealand.....4

Table 1.2: AgResearch commercialised insect biopesticide *Epichloë* species.....7

Table 2.1: Indole-diterpenes analysis by triple quadrupole (TSQ) selected reaction monitoring the following chromatogram segments.....57

Table 2.2: Indole-diterpenes analysis by linear ion trap (LTQxl) selected reaction monitoring the following chromatogram segments.....58

Table 3.1: Genome assemble statistics of AR48 and Fg1.....79

Table 3.2: Mass spectrometry indole-diterpene (IDT) compound profiles of extracts from ryegrass pseudostem infected with different *Epichloë festucae* strains (ppm).....91

Table 3.3: Average intensity of indole-diterpene (IDT) compounds identified in *Penicillium paxilli* wild type, Δ*paxP* (KO-knock-out), and Δ*paxP* complemented with *paxP* from *Penicillium paxilli* or *idtP* from *Epichloë festucae* strains AR48 or Fl1 (ppm) 97

Table 3.4: Average intensity of indole-diterpene (IDT) compounds identified in *Penicillium paxilli* wild type, Δ*paxQ* (KO-knock-out), and Δ*paxQ* complemented with *paxQ* from *P. paxilli* or *idtQ* from *Epichloë festucae* strains AR48 or Fl1 (ppm).....98

Table 3.5: Location of makes caterpillars floppy (*mcf*) in the genomes of fungi outside the *Epichloë* genus using reference genes as location markers.....111

Table 3.6: Characteristics of published Mcf/FitD proteins.....112

Table 3.7: Makes caterpillars floppy (Mcf)/ P. fluorescens insect toxin (FitD) protein domain predictions using online tools that predicts using primary protein structure.....113

Table 3.8: Makes caterpillars floppy (Mcf) and P. fluoresces insect toxin (FitD) protein domain predictions using online tools that predicts using secondary protein structure.....115

Table 4.1: Overall insect bioactivities of *E. festucae* strains against a range of insects using different methodologies.....172
Table 4.2: Comparison of the different parameters between the insect trials used in this study.....173

LIST OF APENDICES

Appendix 1: Alignment of indole-diterpene (IDT) protein sequences from *Epichloë festucae* species.....200

Appendix 2: Unique amino acid changes in indole-diterpene (IDT) IdtP and IdtQ from *Epichloë festucae* strains AR48 and Fg1, when compared in an alignment of corresponding genes in *E. festucae* strains Fl1, E2368, AR1, AR37, and AR5.....202

Appendix 3: Phyre2 analysis of *Epichloë festucae* strains AR48 and Fg1 IdtP sequences aligned to the top 10 hits looking at non-conserved amino acid changes in pocket predictions.....203

Appendix 4: Phyre2 analysis of *Epichloë festucae* strains AR48 and Fg1 indole-diterpene (IDT) IdtQ sequences aligned to the top 10 hits looking at non-conserved amino acid changes in pocket predictions. Underlined amino acid changes are shared with E2368.....205

Appendix 5: *Epichloë festucae* strains AR48 and Fg1 mass spectrometry raw data of new indole-diterpene (IDT) peaks. Initial screening was undertaken using the TSQ (triple-quad) with multiple reaction monitoring (MRM) optimised for the indole diterpene products from *Epichloë* endophytes (parent-18 *m/z* and 130 *m/z* core).....209

Appendix 6: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of paxitriol from *Epichloë festucae* Fl1 infected ryegrass.....212

Appendix 7: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of terpendole E from *Epichloë festucae* Fl1 infected ryegrass.....214

Appendix 8: Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of new indole-diterpene (IDT) compound three from *Epichloë festucae* AR48 and Fg1 infected ryegrass.....218

Appendix 9: *Penicillium paxilli paxP* complementation construct (pSS1, 7930 bp) 219

Appendix 10: *Epichloë festucae* AR48 indole diterpene (IDT) *idtP* complementation construct (pTM06, 11818 bp).....220

Appendix 11: *Epichloë festucae* Fl1 indole-diterpene (IDT) *idtP* complementation construct (pSS56, 5572 bp) 221
Appendix 12: Geneticin construct (pDB49, 7458 bp). NptII (geneticin resistance gene) 222
Appendix 13: *Penicillium paxilli paxQ* complementation construct (pSS2, 8270 bp). Nptll (geneticin resistance gene) 223
Appendix 14: *Epichloë festucae* AR48 indole-diterpene (IDT) *idtQ* complementation construct (pTM07, 11970 bp) 224
Appendix 15: *Epichloë festucae* Fl1 indole-diterpene (IDT) *idtQ* complementation construct (pSS58, 5743 bp) 225
Appendix 16: *Epichloë festucae* Fl1 *perA* complementation construct (pDB05, 16340 bp) 226
Appendix 17: *Penicillium paxilli* (pax) complementation raw data of A) known indole-diterpenes compound and B) unknown indole-diterpene (IDT)-like compounds.....228
Appendix 18: Alignment of makes caterpillars floppy (*mcf*) genes from a range of *Epichloë festucae* strains using the alignment function of Geneious...236
Appendix 19: Alignment of makes caterpillars floppy (Mcf) proteins from a range of *Epichloë festucae* strains using the alignment function of Geneious...239
Appendix 20: Alignment of makes caterpillars floppy (*mcf*) genes from a range of *Epichloë* strains across multiple species using the alignment function of Geneious.....263
Appendix 21: Alignment of makes caterpillars floppy (Mcf) proteins from a range of *Epichloë* strains across multiple species using the alignment function of Geneious.....270
Appendix 22: Alignment of makes caterpillars floppy (Mcf) proteins from a range of fungal species across multiple species using the alignment function of Geneious.....272
Appendix 23: Whole *Epichloë. Festucae* makes caterpillars floppy (*mcf*) gene deletion construct (pTM03, 7489 bp) 273

Appendix 24: 2 kb *Epichloë festucae* Fg1 5' of makes caterpillars floppy (*mcf*) gene deletion construct (pTM05, 8673 bp). Hph (hygromycin resistance gene) 274
Appendix 25: Hygromycin resistant gene construct (pANS7-1, 4777 bp).....275
Appendix 26: PCR confirmation of #117 transformant (Δ*mcf* Fg1/pTM05).....276
Appendix 27: *Epichloë festucae* var *lolii* AR48 makes caterpillars floppy (*mcf*) gene complementation construct (pTM04, 14377 bp). Hph (hygromycin resistance gene).....277
Appendix 28: Hygromycin construct (pDB48, 7702 bp). Hph (hygromycin resistance gene).....278

ABBREVIATIONS

%	Percentage
°C	degrees Celsius
3-GGI	3-geranylgeranyl indole
A600	Absorbance at 600 λ
aa	Amino acid
ACP	Acyl carrier protein domain
ADN	Artificial diet no choice
Amp	Ampicillin
AmpR	Ampicillin resistance
ASW	Argentine stem weevil
AT	Acyltransferase domain
Bcl-2	B-cell lymphoma 2
BH3	Bcl-2 domain three
BLAST	Basic Local Alignment Search Tool
BLASTn	BLAST search against a nucleotide database with a nucleotide sequence
BLASTp	BLAST search against a protein database with a protein sequence
BLASTx	BLAST search against a protein database with a translated nucleotide sequence
Bp	base pairs
BS	Blocking solution
C	Condensation domain
CADN	Cutworm moth caterpillar artificial diet no choice
CC	Cutworm moth caterpillar
cDNA	Complementary DNA
CDTN	Cutworm moth caterpillar detached tiller no choice
CDYE	Czapek Dox yeast extract
CHEF	Contour-clamped homogeneous electric field electrophoresis
CM	Cutworm moth
cm2	centimetres squared
CNF	Cytotoxic necrotizing factors
CoA	Coenzyme A
COMP	Gene complementation
CPD	Cysteine protease domain
CPD 2	Cysteine protease domain two
CPD1	Cysteine protease domain one
CT	Common toxic
CWPC	Cutworm moth caterpillar whole plant choice
CY	Cyclisation domain
CZ	Cell division zone
DH	Dehydratase domains
DMAPP	Dimethylallyl pyrophosphate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease

dNTP	Deoxyribonucleotide triphosphate
DSIR	Department of Scientific and Industrial Research
DTN	Detached tiller no choice
E	Epimerization domain
EAS	Ergot alkaloid
EDTA	Ethylenediaminetetraacetic acid
EZ	Expansion zone
FAD	Flavin adenine dinucleotide
FASTA	Fast-all
Fit	Fluorescens Insect Toxin
FPP	Farnesyl pyrophosphate
g	Gram
g	Acceleration due to gravity
Gen	Geneticin
GenR	Geneticin resistance
GGPP	Geranylgeranyl pyrophosphate
HGT	Horizontal gene transfer
hph	Hygromycin resistance conferring gene
Hyg	Hygromycin
HygR	Hygromycin resistance
IDT	Indole-diterpene
IGP	Indole-3-glycerol phosphate
IPP	Isopentenyl diphosphate
kb	Kilo base pairs
KR	Ketoreductase domains
KS	Keto-synthase domain
L	Litre
LADN	Light brown apple moth caterpillar artificial diet no choice
LB	Lysogeny broth
LBAM	Light brown apple moth caterpillar
LCMS	Liquid-chromatography mass spectrometry
LOL	Loline locus/gene cluster
LTM	Lolitrem locus/gene cluster
M	Moles per litre
MIDAS	Modular Idempotent DNA Assembly System
m/s	Meters per second
Mcf	Makes caterpillars floppy
MEP	2-Methyl-D-erythritol-4-phosphate
mg	Milligram
mins	Minutes
MITEs	Miniature inverted transposable elements
mL	Millilitre
mM	millimoles per litre
mm	Millimetre
MQ	Milli-Q water
mRNA	Messenger RNA

MS	Mass spectrometry
MT	Methyltransferase domains
ng	Nanograms
NMR	Nuclear magnetic resonance
nptII	Geneticin resistance gene
NRPS	Non-ribosomal peptide synthetase
NZGL	New Zealand Genome Limited
OM	Osmoregulation buffer
OX	Oxidation domain
PADN	Porina caterpillar artificial diet no choice
Pax	Paxilline
PC	Porina caterpillar
PC1	Physical containment one
PC2	Physical containment two
PCR	Polymerase chain reaction
PD	Potato dextrose
PDTN	Porina caterpillar detached tiller no choice
PEG	Polyethylene glycol
PER	Peramine
PIPES	Piperazine-N,N'-bis(2-ethanesulfonic acid)
PKS	Polyketide synthase
PM	Porina caterpillar
PM	Porina moth
RG	Regeneration
RNA	Ribonucleic acid
Rnase	Ribonuclease
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT-qPCR	Real time quantitative PCR
RTX	Repeats-in-toxin
SADN	Stem boring fly artificial diet no choice
SBF	Stem boring fly
SD	Secretion domain
SDS	Sodium dodecyl sulfate
SDTN	Stem boring fly detached tiller no choice
sec	Seconds
SM	Secondary metabolite
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
ST	Sorbitol Tris
STC	Sorbitol Tris calcium chloride
SWPC	Stem boring fly whole plant choice
TB	Tris-boric
TBE	Tris-boric acid-EDTA
tBLASTn	BLAST search against a nucleotide database with a protein sequence
TE	Thioesterase domain

Tm	Melting temperature
TMD	Transmembrane domain
UV	Ultra violet
V	Volume
v/v	Volume/volume
w/v	Weight/volume
WA	Water agar
WPC	Whole plant choice
WT	Wild type
Δ	Gene deletion
µg	Microgram
µL	Microliter
µm	Micrometre