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**Hordeae *Epichloë* endophytes and the
formation of synthetic symbioses with
cereal grasses**

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for the degree of
Doctor of Philosophy
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Abstract

This thesis examined two classes of organism that live in symbiosis, grasses and fungi. Specifically it dealt with grasses of the tribe Hordeae (Triticeae) in the subfamily Pooideae and *Epichloë* (*Epichloë* /*Neotyphodium*) fungi of family Clavicipitaceae.

Epichloë endophytes, particularly asexual forms, have important roles in pastoral agricultural systems in the Americas, Australia and New Zealand. Selected strains add value to grass-based forage systems by providing both biotic and abiotic stress resistance. Cereal grasses such as wheat, barley and rye are important to human and animal nutrition and indeed to the foundation and maintenance of Western civilisation. Modern Hordeae cereal grasses such as wheat, barley and rye do not host *Epichloë* endophytes, although grasses of some genera within the tribe, such as *Elymus* and *Hordeum*, do so. Both organism classes, *Epichloë* endophytes and cereal grasses, are of great importance in their own contexts; this research examined the possibility of bringing them together in symbiosis with the ultimate goal of improving cereal production systems.

In this study, a screen of wild *Elymus* and *Hordeum* grasses in Gansu Province, China showed high levels of *Epichloë* infection. A diverse range of fungal genotypes was identified using SSR markers, and chemical screening revealed the production of alkaloid metabolites consistent with the range seen in *Epichloë*-infected pasture grasses of tribe Poae. Importantly, strains were identified that did not produce the mammalian toxins ergovaline or Lolitrem B, although less toxic intermediates such as the indole diterpene paspaline and ergot clavine alkaloids were identified. In addition, strains were identified that produced the insect deterrents/toxins peramine and loline.

Inoculation studies performed in this study demonstrated that cereal grasses could be successfully infected by artificial means using cultured *Epichloë* fungus, although

infected plants generally had poor morphological phenotypes. While alkaloid production of synthetic associations was qualitatively the same as that of native associations, relative quantitative differences were observed between native *Elymus* and synthetic rye. Differences in infection frequencies and host phenotypes were observed between *Epichloë* strains. The choice of *Epichloë* strain used for inoculation profoundly affected the outcome of the symbiosis, ranging from no infection to stunted plants that died prematurely, infected dwarf plants through to normal phenotype plants. Host genotype was also observed to impact infection frequency and phenotype. Family differences in infection phenotype in outcrossing rye suggested a host genetic basis for the observed variation, while population differences in selfing rye indicated that genetics may not have been the sole driver. Consistent phenotypes were observed from the self-fertilizing cereals wheat and barley but, unlike rye, these were not amenable to recurrent selection. Finally, the infection of wheat alien addition/substitution lines showed that there is potential to select wheat-based germplasm with improved phenotypes. Thus, both *Epichloë* genotype and host genotype underpinned successful compatible symbiosis.

This work demonstrated that cereal grasses could be synthetically infected with *Epichloë* and that agriculturally useful metabolites were produced by these symbioses. The manifestation of infection phenotypes highlighted the necessity for careful selection of germplasm for inoculation and a need for selection and breeding of cereal grasses after infection.

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'One thing I have learned in a long life: that all our science, measured against reality, is primitive and childlike -- and yet it is the most precious thing we have.'
Albert Einstein

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Table of Contents

Abstract	I
Acknowledgements	III
Table of Contents	V
List of Figures	VIII
List of Tables.....	X
1 Introduction.....	1
1.1 The fungal symbiont.....	1
1.1.1 <i>Epichloë</i> endophytes.....	1
1.1.2 Anamorph-typified <i>Epichloë</i> (<i>Neotyphodium</i>) endophytes	3
1.1.3 Benefits afforded by endophyte infection	4
1.1.4 Detrimental aspects of endophyte infection	5
1.1.5 Host specificity of <i>Epichloë</i> endophytes in nature	6
1.1.6 Summary of methods to detect <i>Epichloë</i> endophytes	6
1.1.7 Isolation and culture of <i>Epichloë</i> endophytes	7
1.1.8 Artificial infection of grasses with <i>Epichloë</i>	8
1.1.9 Phenotypic and genotypic variation in <i>Epichloë</i> endophytes	9
1.1.10 Loline alkaloids produced by <i>Epichloë</i>	9
1.2 The plant symbiont.....	11
1.2.1 The importance of grasses (family Poaceae)	11
1.2.2 Grass domestication	12
1.2.3 Hordeae.....	12
1.3 Hordeae <i>Epichloë</i> endophytes and the formation of synthetic associations with cereal grasses	17
1.3.1 Natural colonisation of Hordeae with <i>Epichloë</i>	17
1.3.2 The formation of synthetic associations	18
1.4 Background observations that are relevant to this study:.....	18
1.5 Thesis structure.....	19
2 Materials and Methods	21
2.1 Endophyte manipulation	21
2.1.1 Fungal isolation and culture.....	21
2.1.2 Seedling inoculation.....	21
2.2 Growth Media.....	24
2.2.1 Antibiotic ABPDA	24
2.2.2 4% water agar	24

2.3	Endophyte detection	25
2.3.1	Epidermal leaf peel	25
2.3.2	Seed squash	26
2.3.3	Immuno-detection	26
2.4	Simple Sequence Repeats (SSRs), alkaloid determination and beta-tubulin analysis.....	28
2.5	Plant breeding through recurrent selection	28
2.6	Harvest index measurement.....	29
3	Screening wild populations of Hordeae grasses for the presence of <i>Epichloë</i> fungal endophytes.....	30
3.1	Introduction.....	30
3.2	<i>Ex situ</i> germplasm screening	30
3.3	Closest <i>Epichloë</i> progenitor and hybrid status of strains.....	40
3.4	Collection and screening of <i>in situ Elymus</i> germplasm	42
3.5	Screening of <i>Aegilops</i> germplasm.....	48
3.6	Screening Hordeae grasses for <i>Epichloë</i>	49
4	Synthetic symbioses	51
4.1	Introduction.....	51
4.2	Inoculation of rye (<i>Secale cereale</i>).....	51
4.3	Synthetically infected rye are colonised to the leaf tip	57
4.4	Inoculation of selfing rye	57
4.5	Inoculation of hexaploid <i>Triticum</i> with <i>Epichloë</i> isolated from hexaploid <i>Elymus</i>	58
4.6	Inoculation of spring and winter wheat.....	59
4.7	Inoculation of wheat, barley and triticales cultivars with strain AR3060	62
4.7	Inoculation of wheat and barley with loline producing strains from Eurasia	64
4.8	Inoculation of secondary gene pool <i>Hordeum</i> with <i>Epichloë</i> isolated from tertiary gene pool <i>Hordeum</i>	66
4.9	Inoculation of wheat alien addition/substitution lines.....	67
4.10	Infection of <i>Aegilops</i> with <i>Epichloë</i>	68
4.11	The formation of synthetic symbioses between Hordeae grasses and <i>Epichloë</i>	69
5	Host and symbiosis phenotype	70
5.1	Introduction.....	70
5.2	Impact of <i>Epichloë</i> infection on the vegetative size of 'Rahu' rye plants	70
5.3	Infection phenotypes in 'Rahu' rye showed family effects.....	73
5.4	<i>Epichloë</i> -infection affected rye seed but yields similar to un-infected rye could be achieved with recurrent selection	75

5.5	Field grown 'Rahu' AR3002-type produced more heads on plants of similar average height to un-infected 'Rahu'	77
5.6	Harvest Index	78
5.7	<i>Epichloë</i> -infection phenotypes in self-fertile rye.....	81
5.8	Crossing of <i>Epichloë</i> -infected selfing rye	85
5.9	Field grown <i>Epichloë</i> -infected 'Rahu' displayed a larger height range than un-infected 'Rahu' at harvest	87
5.10	Field-grown <i>Epichloë</i> -infected 'Rahu' showed different rust mean lesion scores	88
5.11	Infected rye seed maintained endophyte viability when stored.....	89
5.12	Synthetic Hordeae grass/ <i>Epichloë</i> symbiosis phenotype	90
6	Discussion.....	91
6.1	<i>Epichloë</i> : filamentous fungal endophytes that form symbioses specifically and exclusively with grasses of the Pooideae sub-family.....	91
6.2	Screening of natural populations of Hordeae (Triticeae) grasses <i>Elymus</i> and <i>Hordeum</i> showed presence of <i>Epichloë</i>	93
6.3	<i>Epichloë</i> sourced from <i>Elymus</i> and tertiary gene-pool <i>Hordeum</i> produced similar alkaloids to <i>Epichloë</i> from pasture grasses, but not Lolitrem B.....	94
6.4	Synthetic infections of wheat, barley and rye could be achieved with <i>Epichloë</i> sourced from wild <i>Elymus</i> and <i>Hordeum</i>	95
6.5	Infection of modern cereals with <i>Epichloë</i> sourced from other species resulted in altered morphological phenotypes.....	95
6.6	Desirable <i>Epichloë</i> infection-phenotypes could be selected from within outcrossing populations of rye	96
6.7	Summary and future prospects	97
7	Appendices	100
7.1	Appendix 1 - Annotated SSR dendrogram.....	100
7.2	Appendix 2 – <i>Epichloë</i> strain collection.....	101
7.3	Appendix 3. Grass accessions screened for the presence of <i>Epichloë</i>	104
7.4	Appendix 4. Wheat alien addition/substitution lines inoculated with <i>Epichloë</i> strain AR3060.....	110
7.5	Appendix 5. Harvest Index data for selected spaced plant 'Rahu' rye.....	117
7.6	Appendix 6 Genome Designations in the Triticeae.....	119
8	References.....	125

List of Figures

Figure 1.1 The true grass family Poaceae.....	11
Figure 2.1 Surface sterilisation of seed.	23
Figure 2.2 Inoculation of wheat (<i>Triticum aestivum</i>) with <i>Epichloë</i>	24
Figure 2.3 Aniline blue stained <i>Epichloë</i> in leaf tissue.	25
Figure 2.4 Aniline blue stained <i>Epichloë</i> in seed.....	26
Figure 2.5 Immunoblot of rye (<i>Secale cereale</i>) seedlings.....	28
Figure 3.1 SSR dendrogram.	39
Figure 3.2 Map of China.	42
Figure 3.3 Map of Gansu province.....	43
Figure 3.4 Collection site number 10 Sunan, Gansu Province, China.	47
Figure 3.5 Collection site number 15 Tianzhu, Gansu Province, China.....	48
Figure 4.1 Rye infection phenotypes.	53
Figure 4.2 Infection phenotypes. ‘Rahu’ rye infected with AR3046	56
Figure 4.3 <i>Epichloë</i> growing from the surface sterilised leaf tip on PDA.....	57
Figure 4.4 Un-infected and infected wheat.....	61
Figure 4.5 Un-infected and infected wheat.....	61
Figure 4.6 <i>Epichloë</i> -infected and un-infected triticale.	64
Figure 4.7 <i>Aegilops biuncialis</i> infected with AR3018.	68
Figure 5.1 Pair crosses of plants infected with AR3002-type and AR3007.....	72
Figure 5.2 Plant heights of infected, AR3002-type, ‘Rahu’ rye following several cycles of selection	73
Figure 5.3 ‘Rahu’ rye (<i>Secale cereale</i>) progeny from two individual open-pollinated infected mothers	74
Figure 5.4 Grain of <i>Epichloë</i> -infected (AR3056 - E+) and un-infected (E-) rye (<i>Secale cereale</i>).	75
Figure 5.5 Seed weight of infected (E+), un-infected (E-) and mixed infection individually harvested ‘Rahu’ plants.....	76
Figure 5.6 ‘Rahu’ AR3002-type and E-.....	78
Figure 5.7 KWS selfing rye	82
Figure 5.8 Stature of AR3002-type <i>Epichloë</i> -infected and un-infected selfing rye lines.....	83
Figure 5.9 Floral tiller number and development stage on infected and un-infected selfing rye.....	84
Figure 5.10 Examples of KWS selfing rye infection phenotypes.....	85
Figure 5.11 Schematic of isolation of KWS selfing rye.	86
Figure 5.12 Isolation of selfing rye, infected with <i>Epichloë</i> AR3002-type.....	86
Figure 5.13 Plant height of field-grown ‘Rahu’ infected with strains AR3068 and AR3074 and endophyte-free.....	88

Figure 5.14 Leaf rust (<i>Puccinia</i> spp.) scores – ‘Rahu’ E+ (AR3068, AR3074) and E- (endophyte-free).	89
Figure 5.15 Viability of <i>Epichloë</i> in stored seed.	90

List of Tables

Table 3.1 Species breakdown of grasses examined for the presence of <i>Epichloë</i>	31
Table 3.2 Countries of origin of germplasm screened for <i>Epichloë</i> infection.	34
Table 3.3 Variant groups of <i>Epichloë</i> hosted by <i>Elymus</i> species.....	35
Table 3.4 Chemistry of representative <i>Epichloë</i> variants.....	38
Table 3.5 Closest progenitor and hybrid status of <i>Epichloë</i> strains.....	41
Table 3.6 Infected accessions of <i>Elymus</i> collected in Gansu Province	43
Table 3.7 Accessions collected, number infected and genotypes identified from five regions of Gansu Province	46
Table 3.8 SSR genotypes identified in germplasm from one site (site 10) of Gansu Province collection	46
Table 4.1 'Amilo' rye (<i>Secale cereale</i>) inoculation results (as determined by immunoblot) using a range of hybrid (H) and non-hybrid (NH) <i>Epichloë</i> strains. ND = not determined.	54
Table 4.2 'Rahu' rye (<i>Secale cereale</i>) inoculation results (as determined by immunoblot) using a range of hybrid (H) and non-hybrid (NH) <i>Epichloë</i> strains. ND = not determined.	55
Table 4.3 Inoculation results of orthologous selfing rye lines	58
Table 4.4 <i>Triticum</i> inoculations with <i>Epichloë</i> from hexaploid <i>Elymus</i>	59
Table 4.5 Infection of 'Monad' and 'Savannah' wheat.....	60
Table 4.6 Inoculation of wheat, barley and Triticale seedlings with strain AR3060	62
Table 4.7 <i>Elymus</i> and <i>Hordeum</i> hosts of selected loline producing <i>Epichloë</i> strains.	65
Table 4.8 Inoculation of secondary gene pool <i>Hordeum</i> (<i>H. spontaneum</i> spp. <i>spontaneum</i>) with <i>Epichloë</i> from tertiary gene pool <i>Hordeum</i> species.....	66
Table 5.1 Poly-cross pollen isolations of 'Rahu' AR3002-type and nil	77
Table 5.2 Endophyte-infected 'Rahu' spaced plant trail.....	79
Table 5.3 Harvest Index of spaced plant 'Rahu' rye (<i>Secale cereale</i>)	79
Table 5.4 Harvest index of large and small plants from <i>Epichloë</i> -infected populations of 'Rahu' rye.....	81