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

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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 Hordeeae (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 Hordeeae 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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1.1.1   *Epichloë* endophytes

Epichloë (previously the genera *Epichloë* and *Neotyphodium*) are grass-colonising fungi, belonging to the tribe Balansieae (family Clavicipitaceae) that includes Balansia, Balansiopsis, Myriogenospora, Atkinsonella, Echinodothis and Paraepichloe (Leuchtmann, 2003). Recent changes to nomenclature under the description of 'one fungus, one name' (Hawksworth *et al.*, 2011; Norvell, 2011) requires the use of *Epichloë* to describe both *Epichloë* and *Neotyphodium*. *Epichloë* endophytes colonise a broad range of grasses within the subfamily Pooideae with six of the seven tribes, (as recognised at the time), having one or more genera identified as a host of *Epichloë* (Lane *et al.*, 2000). They are for the most part associated with a narrow taxonomic range of hosts forming a diverse range of symbioses that range through antagonistic/parasitic to mutualistic (Schardl & Clay, 1997; Schardl *et al.*, 2009). They provide documented benefit to some of the grasses that host them (Bouton *et al.*, 2002; Easton, 2007), qualified by the fact that any benefit is dependent on the individual genotype of each host and the environment of the grass/fungus symbiotum (Hesse *et al.*, 2003; Faeth *et al.*, 2004; Hesse *et al.*, 2004; Hesse *et al.*, 2005; Faeth & Hamilton, 2006; Rasmussen *et al.*, 2008).

In natural systems an ecological focus has examined the full range of symbiosis manifestation, while studies of these fungi in agricultural systems has focussed on the mutualistic end of the spectrum. Although the aggregate effect at a population level may be either positive or negative, an examination of individual symbiota has revealed a mosaic of interaction (fungus x host x environment) outcomes.

Although *Epichloë* are obligate symbionts, naturally found only in association with grass hosts, it is possible to isolate and culture them on artificial media in the laboratory (Latch & Christensen, 1985). This ability to isolate and culture the fungi, combined with a method for infecting endophyte-free or endophyte-infected, grasses offers a powerful tool for understanding the nature of the symbiosis and an avenue for the exploitation of these endophytes in agricultural systems (Simpson & Mace, 2012c; Johnson *et al.*, 2013).

Epichloë endophytes have three distinct dispersal mechanism types; type I where stroma are obligatory on infected plants, type II where stroma occur on some inflorescences but not on others and type III (anamorh-typified, formerly *Neotyphodium*) where no stroma is formed (Leuchtmann & Clay, 1997). *Epichloë* endophytes are intercellular colonisers that either give rise to no visible symptoms of infection, or symptoms are delayed until flowering commences. During flowering, stromata formation can give rise to 'choking' of the inflorescence which is subsumed by the stromal mycelium of the fungus (Craven *et al.*, 2001). With the exception of the period when stromata are formed in type I and type II infections, the colonisation of grasses with *Epichloë* endophytes is asymptomatic and as such, infected plants are not distinguishable from uninfected plants. In type III infections, endophyte-infected and endophyte-free plants are indistinguishable for the duration of the lifecycle of the symbiosis.

Colonisation by *Epichloë* can enhance host plant protection against both vertebrate and invertebrate herbivores *via* a number of alkaloidal secondary metabolites, the most well studied of which are; ergovaline, lolitrem B, peramine and lolines (Clay, 1989, 1993; Bush *et al.*, 1997; Schardl, 2001; Giménez *et al.*, 2007; Kuldau & Bacon, 2008). Ergovaline is an ergot alkaloid and its biosynthesis involves a complex gene cluster (Fleetwood *et al.*, 2007). Lolitrem B forms part of a structurally diverse group of indole-diterpene mycotoxins and also requires a complex gene cluster for biosynthesis (Young

et al., 2006). Peramine is a pyrrolopyrazine, the putative product of a two-module non-ribosomal peptide synthetase (Tanaka *et al.*, 2005) and lolines are comprised of a saturated 1-aminopyrrolizidine-ring system and their biosynthesis involves two homologous gene clusters LOL-1 and LOL-2 (Spiering *et al.*, 2005). Due to their importance in synthetic symbioses anticipated for Hordeeae cereal grasses, lolines will be discussed in more detail later. These compounds are produced in various combinations throughout the *Epichloë* including the asexual, anamorph-typified species.

1.1.2 Anamorph-typified *Epichloë* (*Neotyphodium*) endophytes

Anamorph-typified *Epichloë* endophytes are asexual derivatives of *Epichloë* that infect a number of cool season grasses of the order Pooideae (Clay, 1993; Schardl, 1996; Christensen *et al.*, 2002). The anamorph-typified *Epichloë* form asymptomatic and, for the most part, mutualistic symbioses with their hosts, and transmit vertically *via* host seed colonisation (Schardl *et al.*, 1997). Although anamorph-typified *Epichloë* have been widely described as mutualistic, this claim has been challenged; it has been suggested that, although the interaction with agronomic grasses such as perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*) tends toward a tight mutualism, within the wider context, grass host/endophyte interactions range between antagonism and mutualism (Saikkonen *et al.*, 1998; Faeth, 2002; Easton, 2007).

The genus *Neotyphodium* was previously known as *Acremonium*. A review of the taxonomy was made following an examination of the molecular phylogeny of *Acremonium*. Glenn et al. (1996) used parsimony analysis of 18S rDNA sequences of a number of fungal orders including Clavicipitales to reclassify the anamorphs of *Epichloë* and related mutualists, forming the genus *Neotyphodium* (Glenn *et al.*, 1996). This nomenclature arose from the fact that Diehl (1950), as a convention of convenience,

applied Typhodium as a form genus and used the term typhoidal when referring to the anamorph (asexual stage) of *Epichloë*.

Many anamorph-typified *Epichloë* are hybrids with ancestors among two or more biological species of *Epichloë*. Eleven distinct biological species (mating populations) of *Epichloë* have been described (Moon *et al.*, 2002; Moon *et al.*, 2004; Kuldau & Bacon, 2008), most of which exhibit host specificity for groups of related grass genera; a recent re-alighment recognises 10 teleomorph-typified and 24 anamorph-typified species (Leuchtmann *et al.*, 2014).

Many *Epichloë* species and all of their asexual relatives are transmitted vertically *via* the seeds of infected plants (Schardl, 1996). In the vertical transmission route of asexual and pleiotropic *Epichloë* endophytes, the fungus invades the developing ovule and ultimately the embryo of mature seeds (Philipson & Christey, 1986). In this clonal, highly efficient means of propagation, nearly 100% of seeds from infected mother plants transmit the endophyte (Siegel *et al.*, 1984).

1.1.3 Benefits afforded by endophyte infection

The symbioses that anamorph-typified *Epichloë* form are mutualistic in that both the fungus and the host grass benefit from the association. The fungus benefits from a biological niche with few if any competing organisms and a potentially ready source of nutrients in the host apoplastic fluid along with a mechanism for vicarious dispersal *via* the host seed. The host benefits from the range of secondary metabolites the fungus produces in the form of alkaloids, many of which have individual and/or multiple activities against different classes of organisms.

In *Epichloë festucae* var *lolii*/*Lolium perenne* associations in New Zealand, insect pest resistance, *via* the production of peramine, ergovaline and indole diterpenes (IDTs), is the primary advantage effected. Peramine is associated with resistance to the pasture pest

Argentine Stem Weevil (*Listronotus bonariensis*) (Prestige *et al.*, 1991) while ergovaline is associated with resistance to African Black Beetle (*Heteronychus arator*) (Ball *et al.*, 1997). The tall fescue endophyte *E. coenophiala* (*N.coenophialum*) confers its primary advantage *via* drought resistance and is capable of extending the southern range limit of tall fescue grasses in agricultural areas of the southern regions of North America. The research into this phenomenon has examined both direct physiological effects of the endophyte symbiont on the physiology of the host plant affecting stomatal conductance and osmotic adjustment (Elm & West, 1995), including transcription profiling (Zhou, 2014), and indirect effects *via* differences in insect predation and nematode populations affecting the host plant through the production of lolines (West *et al.*, 1987). Indole diterpenes, along with their proven animal toxicity, have been posited as compounds with activity against invertebrate pests (Young *et al.*, 2009).

1.1.4 Detrimental aspects of endophyte infection

In addition to the positive aspects of *Epichloë* infection of grasses in New Zealand pastures, secondary metabolites produced by the fungus can result in animal toxicoses. Ryegrass staggers is caused by the neurotoxin Lolitrem B, one of several alkaloid metabolites produced by endophyte-infected perennial ryegrass of the New Zealand ecotype (Fletcher & Harvey, 1981). The connection between this fungus and ryegrass staggers was suggested by Fletcher and Harvey (Fletcher & Harvey, 1981) when they correlated the level of endophyte infection with scores for severity of the livestock condition in grazing hoggets. Regional and sporadic outbreaks can be explained by the fact that alkaloids are produced by the fungus differentially both *in planta* and in culture (Blankenship *et al.*, 2001; Tanaka, *et al.*, 2005; Young, *et al.*, 2006) and that toxin production is affected by the genotype of the host plant and the environment (Easton *et al.*, 2002). Additionally, the ergot alkaloid ergovaline is produced in endophyte-infected

New Zealand ecotype ryegrass and can result in low live weight gains and general ill-thrift of grazing animals (Fletcher *et al.*, 1999).

1.1.5 Host specificity of *Epichloë* endophytes in nature

The associations that the various *Epichloë* species form are host specific. For example *E. festucae* var *lolii* (*N.lolii*) specifically colonises perennial ryegrass (*Lolium perenne*), *E. coenophiala* colonises tall fescue (*L. arundinaceum* syn. *Schedonorus phoenix* syn. *Festuca arundinacea*), *E. uncinata* (*N.uncinatum*) colonises meadow fescue (*Festuca pratensis*) and *E.occultans* (*N. occultans*) colonises annual grasses such as *L. multiflorum*. It is thought that speciation of the host has progressed alongside that of the symbiont fungus, with the resulting co-speciation manifesting as host specificity (Schardl, *et al.*, 1997). It is suggested that multiple infections from sexual *Epichloë* spp. have given rise to hybrid asexual endophyte species (Schardl *et al.*, 1991) that have become trapped in their host species. Schardl and co-workers have shown, using molecular techniques, that multiple copies of *tub2* genes are present in many anamorph-typified *Epichloë;* suggesting that the different species have developed by super-infection and hybridisation within their host grasses (Schardl & Clay, 1997; Moon*, et al.*, 2004). Furthermore the hybrid status of *Epichloë* is reflected in genome size (Kuldau *et al.*, 1999).

1.1.6 Summary of methods to detect *Epichloë* endophytes

Various methods are employed to either detect or directly observe *Epichloë* endophytes in plant tissues. A long-used method for observing endophyte hyphae in fresh tissue involves removing the single cell layer of tissue that forms the epidermis on the adaxial surface of the host leaf sheath and mounting this on a microscope slide with aniline blue stain. Aniline blue stains the cytoplasmic contents of intact hyphae. Using this method the endophyte hyphae appear as long, septate, even-width filaments that run parallel to the leaf axis.

Endophytes can also be indirectly detected using immunological techniques. Two methods commonly used are a microtitre plate based enzyme-linked immunosorbance assay (ELISA) and an antigen binding matrix dot-blot or immuno-blot (Musgrave, 1984; Simpson *et al.*, 2012b). Both of these systems require the production of antibodies raised against the endophyte fungus. This is done by eliciting an immune response in small mammals using a preparation of cultured endophyte. Immunoglobulins are purified from serum removed from inoculated animals (Musgrave, 1984). The use of ELISA facilitates not only endophyte detection but also a degree of quantification. The immuno-blot approach is a quick and simple method of detecting endophyte but it does not allow any form of quantification (Gwinn *et al.*, 1991; Hill *et al.*, 2002).

These two techniques will detect viable endophyte in fresh tissue. It is also possible to detect endophyte in seed, but the assays give no indication of fungus viability. Mycelium can be observed directly by staining seed tissues with aniline blue and observing them under a compound light microscope. Endophyte mycelium can be readily detected by removing tissue that includes the aleurone cells of the seed. Hyphae are present in large numbers at the interface of the endosperm and scutellum of infected seed (Philipson & Christey, 1986). Seed can also be assayed using an immuno-technique. Antigen is obtained from seed by soaking in dilute sodium hydroxide and extracting onto a membrane placed on a sponge saturated with an extraction buffer (Hill, *et al.*, 2002).

1.1.7 Isolation and culture of *Epichloë* endophytes

In addition to staining and immunological detection techniques, endophytes can be detected by isolation from either fresh tissue or seed. *Epichloë* endophytes are biotrophic in nature and can be thought of as fungi trapped within the grass host plant (Schardl & Clay, 1997). In the laboratory however it is possible to isolate and culture them (Latch & Christensen, 1985). Isolation involves the surface sterilisation of fresh pseudostem tissue

excised from an infected plant that is plated onto a suitable solid medium. After three to seven days (sometimes longer depending on the endophyte strain) hyphal filaments will emerge from the tissue and form colonies on the agar that are visible to the naked eye after 2 to 3 weeks. The colonies that develop can be used to establish endophyte infections in uninfected potential hosts *via* artificial inoculation.

1.1.8 Artificial infection of grasses with *Epichloë*

In nature, the anamorph-typified *Epichloë* rely on host seed production to disseminate. This reliance on vertical transmission contrasts with the sexual or teleomorph-typified *Epichloë* that can colonise uninfected hosts horizontally *via* ascospores. It is possible however to infect endophyte-free host grasses artificially in the laboratory and these infections can result in plants that are morphologically indistinguishable from uninfected plants. In this way the anamorph-typified *Epichloë* can be established in novel hosts (Latch & Christensen, 1985).

The ability to perform such artificial infections is central to attempts to solve the problem of animal toxicity due to alkaloids while retaining the host protection properties of the endophyte symbiosis. Collections of fungal endophytes have been screened according to their alkaloid profile using HPLC and strains that do not produce any of the known mammalian toxins have been isolated. These fungi are then used to infect elite pasture grass germplasm to produce toxin free pastures that retain resistance to invertebrate pests Examples of of commercially available *Epichloë* endophytes include the ryegrass strain AR1 that produces the insect active metabolite peramine, with no production of the animal toxins lolitrem B and egovaline and AR584 that produces lolines but no ergovaline (Easton, 2007; Johnson, *et al.*, 2013).

1.1.9 Phenotypic and genotypic variation in *Epichloë* endophytes

There are many strains of *Epichloë* endophyte. These strains manifest differences not only between broad taxonomic groupings and species (Moon*, et al.*, 2004; Hettiarachchige *et al.*, 2015) but also within species (Moon *et al.*, 1999; Van Zijll De Jong *et al.*, 2003; Card *et al.*, 2014). This is demonstrated also in a range of alkaloid and isozyme phenotypes and differences in colony morphologies of the endophytes in culture (Christensen *et al.*, 1993). The non-hybrid *E. festucae* var *lolii,* for example, shows pronounced colony morphology variation with mycelium ranging from sparse to abundant, felted, cottony or aggregated into erect tufts; colour from white to brown; shape from flat, raised, domed, smooth, convoluted, crusted or brain-like and texture from waxy through to yeast-like, dry or cottony (Christensen *et al.*, 1991).

1.1.10 Loline alkaloids produced by *Epichloë*

Some *Epichloë* fungal endophytes are capable of producing loline alkaloids that have powerful insecticidal activity (Spiering *et al.*, 2008). The common lolines produced by *Epichloë* are saturated pyrrolizidine alkaloids, specifically; loline, norloline, N-methylloline, N-formylnorloline, along with N-formyl loline (NFL), N-acetyl loline (NAL) and N-acetyl norloline (NANL) (Schardl*, et al.*, 2009). The presence of lolines can benefit the host plant through anti-insect activity, either by deterring insects or *via* insecticidal effects, acting as both metabolic toxins and as feeding deterrents depending on the species of insect (Siegel *et al.*, 1990; Bush*, et al.*, 1997). Effects can be seen with a broad array of insects, e.g. NFL has been shown to have effects ranging from behavioural changes to mortality on a range of insects including beetles and flies, in addition to cat fleas and cockroaches (Riedell *et al.*, 1991; Dahlman *et al.*, 1997; Dougherty *et al.*, 1999; Wilkinson *et al.*, 2000). Within a pasture setting, lolines impact pests such as bird-cherry oat aphid (*Rhopalosiphum padi*), greenbug (*Schizaphis*

graminum), Japanese beetle (*Popillia japonica*), fall armyworm (*Spodoptera frugiperda*), and European corn borer (*Ostrinia nubilalis*) (Schardl *et al.*, 2007). Grasses hosting loline producing *Epichloë* strains include representatives of tribes Poeae (*Lolium*, *Poa* and *Festuca*), Triticeae (=Hordeeae) (*Hordeum*), Aveneae (=Poeae) (*Agrostis*, *Echinopogon*) and Stipeae (*Achnatherum*) (Schardl, *et al.*, 2007). Documented hosts of loline producing strains originate in North America, Europe, North and South Africa, Asia and Australasia. Two *Epichloë* (non-hybrid) species produce lolines, *E. amarillans* and *E. festucae*. Hybrid *Epichloë* that produce lolines align with two or more of the following contributing *Epichloë* species; *E. festucae*, *E. typhina*, *E. baconii* and *E. bromicola* (Schardl, *et al.*, 2007; Schardl, 2010). Given the potential positive effects that lolines might bring to cereal production systems, coupled with the absence of mammalin toxicity (Finch *et al.*, 2016) *Epichloë* strains that produce lolines will be targeted in the studies that follow.

1.2 The plant symbiont
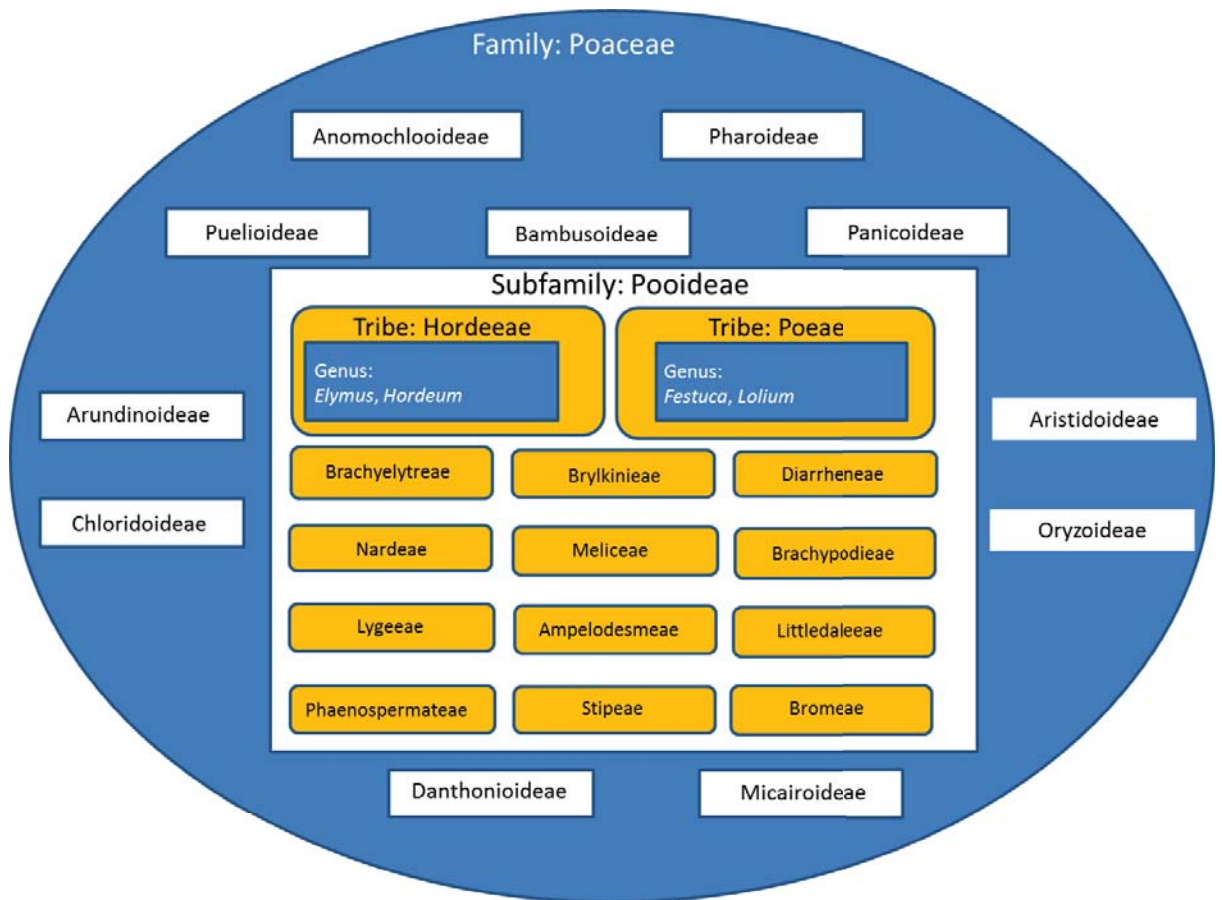
Figure 1.1 The true grass family Poaceae.Twelve subfamilies are represented by white boxes.The subfamily Pooideae, that hosts *Epichloë*, is enlarged and populated with 14 tribes including Hordeeae (Triticeae), the focus of this study; and Poeae, that includes genera such as *Festuca* and *Lolium* that provide a case study for the benefits of *Epichloë* infection for cool-season grasses. Modified from Simpson *et al* (2014) (Appendix 7).

1.2.1 The importance of grasses (family Poaceae)

The grass family, Poaceae (Fig.1), previously known as Gramineae, consists of 12 subfamilies and is one of the four largest families of flowering plants with around 900 genera and 10,000 species (Tzvelev, 1989; Soreng *et al.*, 2015). The family is thought to have diverged from an ancestral progenitor 50 to 70 million years ago (Kellogg, 2001; Huang *et al.*, 2002; Levy & Feldman, 2002). Grasses are very important to mankind, instrumental in the transition ca. 12,000 years ago, from hunting and gathering

communities to agricultural based societies (Salamini *et al.*, 2002). In addition to their economic importance, they are of substantial importance in ecosystems, playing important roles in the composition and functioning of natural plant communities. Grasses provide over half of the world's caloric intake (Kellogg & Buell, 2009). Of particular importance in this regard are cereal grasses in the tribe Hordeeae (=Triticeae) with crops such as wheat, barley and rye forming essential components of both human and domestic animal nutrition (Feuillet & Muehlbauer, 2009).

1.2.2   Grass domestication

Until approximately 12,000 years ago humans did not actively cultivate plants. Since this time crop plants have been cultivated either as wild or domesticated (or under domestication) forms. The evidence suggests that western agriculture originated in the Fertile Crescent in the Near East, including areas of Israel, Jordan, Syria, Iraq and Iran with the wild progenitors of modern cereal species such as wild wheats (*Triticum urartu*, *T. boeoticum* and *T. dicoccoides*), wild barley (*Hordeum spontaneum*) and wild rye (*Secale vavilovii*) (Salamini*, et al.*, 2002). There is some suggestion however that the domestication process began much earlier with evidence of cereal grain processing from a 23,000 year old campsite at Ohalo II on the southwestern shore of the Sea of Galilee in Israel (Nadel *et al.*, 2012).

1.2.3   Hordeeae

The taxonomy and nomenclature around the Pooideae grass tribe Hordeeae is well known for its complexity and the disagreement that surrounds its delimitation and specification (Dewey, 1983). The tribe designation 'Hordeeae' has been adopted for use in this thesis following the Nomenclatural section of the 17th International Botanical Congress, held in Melbourne, Australia in 2011, where changes were made to the International Code of Botanical Nomenclature (IBCN) (Hawksworth*, et al.*, 2011; Norvell, 2011).The code

iself changed name.The code is now known as the International Code of Nomenclature for algae, fungi and plants (ICN). Amongst other changes was one to accept Martinov's names as having been validly published. Adopting this requires changing of the name of the tribe that includes wheat and barley back to Hordeeae, the nomenclature continues to be debated however (McNeill *et al.*, 2012; Barkworth & Von Bothmer, 2014). The number and names of genera vary over the different taxonomic treatments but generally there are around 350 species included in the tribe (Barkworth & von Bothmer, 2009) with contemporary treatments grouping these species within about 30 genera. Cytotaxonomic description of tribe Triticeae (= Hordeeae) as promoted by Löve (1982) proposes a generic taxonomy on the basis of a rigorous genomic concept that dictates that each genome (or haplome) or combination thereof should be the base to define a genus. Löve's "Conspectus of the Triticeae" (Löve, 1984) is "a taxonomical and nomenclatural survey of the more than 500 biological taxa of the Triticeae tribe of grasses in a system of thirty-seven genomically defined genera based on twenty-three single-haplome taxa". It assigns letter designations to genomes and combinations of genomes in Triticeae grasses (Löve, 1982; Dewey, 1984; Löve, 1984; Yen *et al.*, 2005). See also appendix 6.

1.2.3.1 *Elymus*

Elymus is the most specious genus of the Hordeeae with around 150 species documented (Dewey, 1984; Okito *et al.*, 2009; Wang & Jensen, 2009). The genus is widely distributed and can be found in most temperate areas of the world including Europe, Asia, North America, South America, New Zealand, Australia and Northern Africa (Jensen & Salomon, 1995) . All are polyploid perennials with three quarters being tetraploid species consisting of StH and StY genome combinations (Dewey, 1984; Lu *et al.*, 1995). The StY genome species that are distributed in Asia have been delimited as *Roegneria* species (Baum *et al.*, 1991; Baum *et al.*, 2003). The St genome is derived from *Pseudoroegneria*,

the H from *Hordeum* and the Y genome from a supposed diploid progenitor that remains unknown (Hodge *et al.*, 2010; Mason-Gamer *et al.*, 2010a). Other possibilities in *Elymus* include combinations involving the P genome from *Agropyron* and the W genome from *Australopyrum* (Jensen, 1990; Xu & Ban, 2004). A study examining the elongation factor G (*EF-G*) gene sequence demonstrated that the Y genome has sequence similarity to the W genome, found specifically in Australasian Hordeeae (Sun & Komatsuda, 2010). All *Elymus* contain at least one set of *Pseudoroegneria* derived St genomes forming allopolyploids with the other Hordeeae genomes mentioned above. *Pseudoroegneria* is the probable maternal genome donor to both StY and StH *Elymus* species (Mason-Gamer *et al.*, 2002; Hodge*, et al.*, 2010). There is evidence for a strong preference for cytoplasmic DNA inheritance from *Pseudoroegneria*, and there is a suggestion that hybridisations having the St-containing parent as the female may be more successful (Redinbaugh *et al.*, 2000). There are approximately forty tetraploid *Elymus* with the StY genomes. Their natural distribution is restricted to Asia, with the exception of *E. panormitanus* which is also found in South East Europe (Lu & Salomon, 1992). The StH tetraploids number around fifty species distributed throughout much of North America, Europe and western Asia with evidence that the North American and Eurasian species arose independently (Jaaska, 1992; Linde-Laursen *et al.*, 1994; Jaaska, 1998; Sun *et al.*, 2008) but also that the group originated exclusively in North America (Mason-Gamer *et al.*, 2010b). Although tetraploids predominate in *Elymus* there are approximately 20 hexaploid species, occurring primarily in Eurasia, (mostly eastern and central Asia and the Himalayas) and a few octoploids which occur mainly in North America (Dewey, 1984; Lu & Von Bothmer, 1992). The genomic constitutions of around 40% of *Elymus* species are unknown and some remain to be verified (Okito*, et al.*, 2009).

1.2.3.2 *Hordeum*

Hordeum comprises around 30 species, with the unifying morphological character being single-flowered spikelets borne three together at the rachis node, (so called triplets) (von Bothmer, 1992). *Hordeum* is unusual among the Hordeeae as it consists of both annual and perennial species. Both cultivated barley, *H. vulgare*, and its wild progenitor *H. spontaneum* are diploid species (2n=2x=14), while other species are tetraploid (2n=4x=28) and hexaploid (2n=6x=42) (Komatsuda *et al.*, 1999). The genus originated around 12 million years ago in western Asia and currently can be found in central Asia, Europe and the Americas as well as South Africa. The genus is notably absent from Australasia. The H-genome group of the genus went through rapid radiation about 2.5 million years ago in South America and Asia (Blattner, 2004, 2006; Jakob & Blattner, 2006). Three Asian diploid H-genome species, *H. brevisubulatum* 2x, *H. bogdanii* and *H. roshevitzii* show a commonality in that all have only a single 12-10kb fragment when examined for rDNA-RFLP; contrasting with American diploid *Hordeum* species that have two or more fragments (Taketa *et al.*, 2005).

1.2.3.3 Barley

Barley (*H. vulgare*) was domesticated, according to archaeological evidence, from its wild relative *H. spontaneum* around 10,000 years ago (Badr *et al.*, 2000). Although the near East Fertile Crescent has long been recognised as the area where barley was domesticated, recent molecular evidence suggests that a second domestication occurred to the east, in Central Asia and Tibet, that has contributed to Central and East Asian barleys of today (Ren *et al.*, 2013).

1.2.3.4 Wheat

The ploidy level of domesticated wheat species range from diploid (2n=14) to hexaploid (6n=42). Diploid wheat, Einkorn (*Triticum monococcum*), the earliest form of cultivated

wheat, has an AA genome. Crosses of the tetraploid species, *T. turgidum* ssp. *dicoccum* (AABB) with the wild diploid species *Aegilops tauschii* (DD) have formed hexaploid wheat *T. aestivum* (AABBDD). (Salamini*, et al.*, 2002; Feuillet *et al.*, 2008).

1.2.3.4 1 Alien addition lines involving wheat

Wild relatives of wheat are recognised as having potential as sources of genes for improving wheat performance. It is possible to add or substitute entire chromosomes, chromosome areas or chromatin segments. The rearrangement of wheat chromosomes in this way has constituted an important aspect of wheat improvement for over 50 years (Graybosch, 2001). Transfer is effected by producing an amphidiploid, a hybrid between the two species having at least one complete diploid set of chromosomes from each species, or a partial amphidiploid, and then producing individual chromosome addition lines. Following this, the centric breakage fusion tendency can be exploited to transfer a whole alien chromosome arm. Strategies can also be deployed for transferring alien segments that are smaller than complete chromosome arms (Qi *et al.*, 2007). The availability of genetic diversity within populations provides the basis for selection for desirable agronomic traits. Where genetic diversity is limited, such as in highly selected and inbred wheat lines, the introduction of alien chromosomes from related species offers a means to increase the genetic base of the population and broaden the possibilities for the selection of desirable traits. Alien chromosome introgressions from rye to wheat have been used to confer resistance to pathogens and insects (Driscoll & Jensen, 1964; Stewart *et al.*, 1970) reviewed by Sharma and Gill (Sharma & Gill, 1983). The stability of wheat/rye chromosome substitution lines can vary depending on the base cultivars used (Alkhimova *et al.*, 1999).

1.2.3.5 Rye

Rye is less important as a cereal crop globally than wheat but it does have regional significance, for example, as a bread cereal in Northern Europe (Merker, 1992). One of the features of rye that make it important in some regions is its ability to grow in poor soils and withstand severe winters. Leading rye producers include former Czechoslovakia and U.S.S.R countries, Poland, Germany, Hungary, Canada and the United States (Morey & Barnett, 1980). Rye is a functional annual with a diploid genome size of 8 Gb (Morey & Barnett, 1980; Feuillet *et al.*, 2012), that is thought to have diverged from a common ancestor of wheat six million years ago (Huang*, et al.*, 2002). The domestication process for rye is not as well understood as that of other cereal crops; it is likely that it was cultivated before its domestication and may have first appeared as a tolerated weed (Feuillet*, et al.*, 2008). It has a genome constitution RR with two diploid progenitors *Secale vavilovii* and *S. montanum* posited (Stutz, 1972), although a phylogenetic study, using polymorphic AFLP loci showing high resolution genetic relationships among rye taxa, failed to show close relationship of *S. vavilovii* to *S. cereale* (Salamini*, et al.*, 2002; Chikmawati *et al.*, 2005). When analysed using isozyme data, *S. montanum* populations could not be distinguished from *S. cereale* populations (Vences *et al.*, 1987).

1.3   Hordeeae *Epichloë* endophytes and the formation of synthetic associations with cereal grasses

1.3.1   Natural colonisation of Hordeeae with *Epichloë*

There are several reports of *Epichloë* naturally colonising Hordeeae grasses. A study examining 21 accessions of *Hordeum* held at the National Small Grains Collection (NSGC), Idaho, USA found three infected accessions, namely *H. bogdanii*, *H. brevisubulatum* ssp. *violaceum* from Asia and *H. comosum* from South America (Wilson *et al.*, 1991; Wilson, 2007). An endophyte designated *E. elymi* has been identified in four different *Elymus* species in the United States of America, *E. candadensis*, *E. hystrix*, *E.*

villosus and *E. virginicus* (Kuldau & Bacon, 2008). In China, many infected Hordeeae have been reported and some new endophyte species described; *Elymus* species such as *E. dauhuricus*, *E. excelsus*, *E. nutans*, *E. breviaristatus*, *E. glaucus* and *E. sibiricus,* infected with *Epichloë*, have been identified along with a number of species of *Roegneria*, *Agropyron*, *Elytrigia* and *Leymus* (Li *et al.*, 2006; Wei *et al.*, 2006; Yan *et al.*, 2009; Kang *et al.*, 2011; Zhu *et al.*, 2013).

1.3.2   The formation of synthetic associations

Given the documented benefit of *Epichloë* in agricultural systems, with a great number of *Epichloë* studies focused on pasture grasses, and the undoubted importance of cereal grasses, the question examined by this thesis is "what are the possibilities for *Epichloë* endophytes in modern cereal grasses?" Specifically, what is the extent of natural infection of Hordeeae? How genetically diverse are the *Epichloë* fungal strains? What is the extent and nature of potential synthetic associations of these fungi with modern cereal grasses and what are the possibilities, using plant breeding and selection, to select for desirable symbiosis traits? In addressing these questions, knowledge of the genetics of the host grasses that form the source of fungal strains along with the target novel hosts is important as is knowledge of the host range and closest known progenitors of the fungi involved.

1.4   Background observations that are relevant to this study:

- *Epichloë* fungal endophytes provide well documented benefit to grasses deployed in pastoral agricultural systems.

- This class of fungal endophyte occurs naturally in the grass tribe Hordeeae (Triticeae) to which the major cereal grasses wheat, barley and rye belong.

- Both fungal and host genetics contribute to the symbiosis phenotype.

Based on these observations, this thesis will address the following research questions:

i. Is it possible to form stable artificial symbioses between *Epichloë* endophytes sourced from wild grasses and major cereal grasses of the Hordeeae tribe?

ii. Can functional differences be detected in genetically distinct *Epichloë* endophyte strains?

iii. Does genetics of the potential host affect infection and the nature of the symbiosis formed?

iv. Can host genetics be manipulated to maximise compatibility of the symbiosis

1.5   Thesis structure

There are three key components of this research. (i) Endophyte discovery, (ii) the formation of synthetic symbioses and (iii) selection and breeding of infected germplasm.

Endophyte discovery is covered in chapter three. This section describes the sourcing of wild germplasm, both from existing collections (*ex situ*) and from a collection undertaken specifically for this research (*in situ*). Screening for *Epichloe* infection is described and infected plants are retained for genetic and chemical characterization of the fungus. Selected strains are used in the next phase, the formation of synthetic symbioses.

The formation of synthetic symbioses is described in chapter four. Here a laboratory-based inoculation procedure, described in chapter two, is used to attempt infection of novel potential hosts. In the context of this thesis, *Epichloe* sourced from wild Hordeeae grasses are inoculated into modern cultivated Hordeeae cereal grasses including wheat,

barley and rye. Where infection is achieved the phenotype of infected plants is described, these plants then form the material for the selection and breeding of infected germplasm.

Selection and breeding of infected germplasm is outlined in chapter five.The possibility of manipulating symbiosis phenotype through selective crossing is explored. The outcrossing cereal rye (*Secale cereale*) provides a system to explore this aspect of the biology of novel synthetic *Epichloe*/cereal grass symbioses. Phenotype selection in a self fertilizing system is also examined using a hybrid rye cytoplasmic male sterile (cms) population.

These three themes of research combine providing a co-ordinated scheme exploring the possibilities of deploying *Epichloe* endophytes in cereal grasses with the ultimate aim of improving production systems.

2 Materials and Methods

2.1 Endophyte manipulation

The ability to detect the presence of *Epichloë* in host-grass tissues and to isolate and culture them is fundamental to any effort to characterise and then deploy these fungi in novel hosts through the establishment of synthetic symbioses. *Epichloë* are obligate endo-symbionts in nature, however they can be isolated and cultured in the laboratory using standard microbiological techniques.

2.1.1 Fungal isolation and culture

Fungus was isolated from endophyte-infected plants following surface sterilisation of plant tissue as described by Christensen et al. (Christensen*, et al.*, 2002). Tillers were removed from either wild or synthetically infected Pooideae grasses by cutting at the base and trimming to ca. 5cm before surface sterilising. Sectioned tillers were surface sterilised by quick rinse with 96% ethanol and a 1 minute soak in a sodium hypochlorite solution (10% Janola: 42g/L NaOCl domestic bleach), followed by rinsing twice in sterile water. Tillers were sectioned transversely, sheath rings were separated and plated on to antibiotic potato dextrose agar (ABPDA - see 2.2.1). Plates were incubated in the dark at 22-25°C for 3-5 weeks.

2.1.2 Seedling inoculation

Seed was surface sterilised and inoculated as described by Latch and Christensen (Latch & Christensen, 1985). Seed was surface sterilsed by immersion in a 50% sulphuric acid solution for 15min and rinsed five times with tap water, immersed in a 10% domestic bleach (Janola) solution for 15min followed by two rinses in sterile water. Seed was dried in a laminar flow cabinet on sterile Whatmann filter paper before arranging on 4% water

agar Petri plates (Fig. 2.1 and section 2.2.2). Plated seed was germinated in the dark at 22-25°C for 5-7 days. Resulting etiolated seedlings were inoculated by placing cultured mycelium into a slit cut in the base of the seedling plant (Fig. 2.2) before being returned to the dark incubator for 7 days. Following this incubation plates were placed under white fluorescent lights at ambient for at least 7 days before removing seedlings and planting them in commercial potting mix and growing them in a glasshouse under natural light and temperature. Plants were grown for ca. 6 weeks before identifying infected individuals.
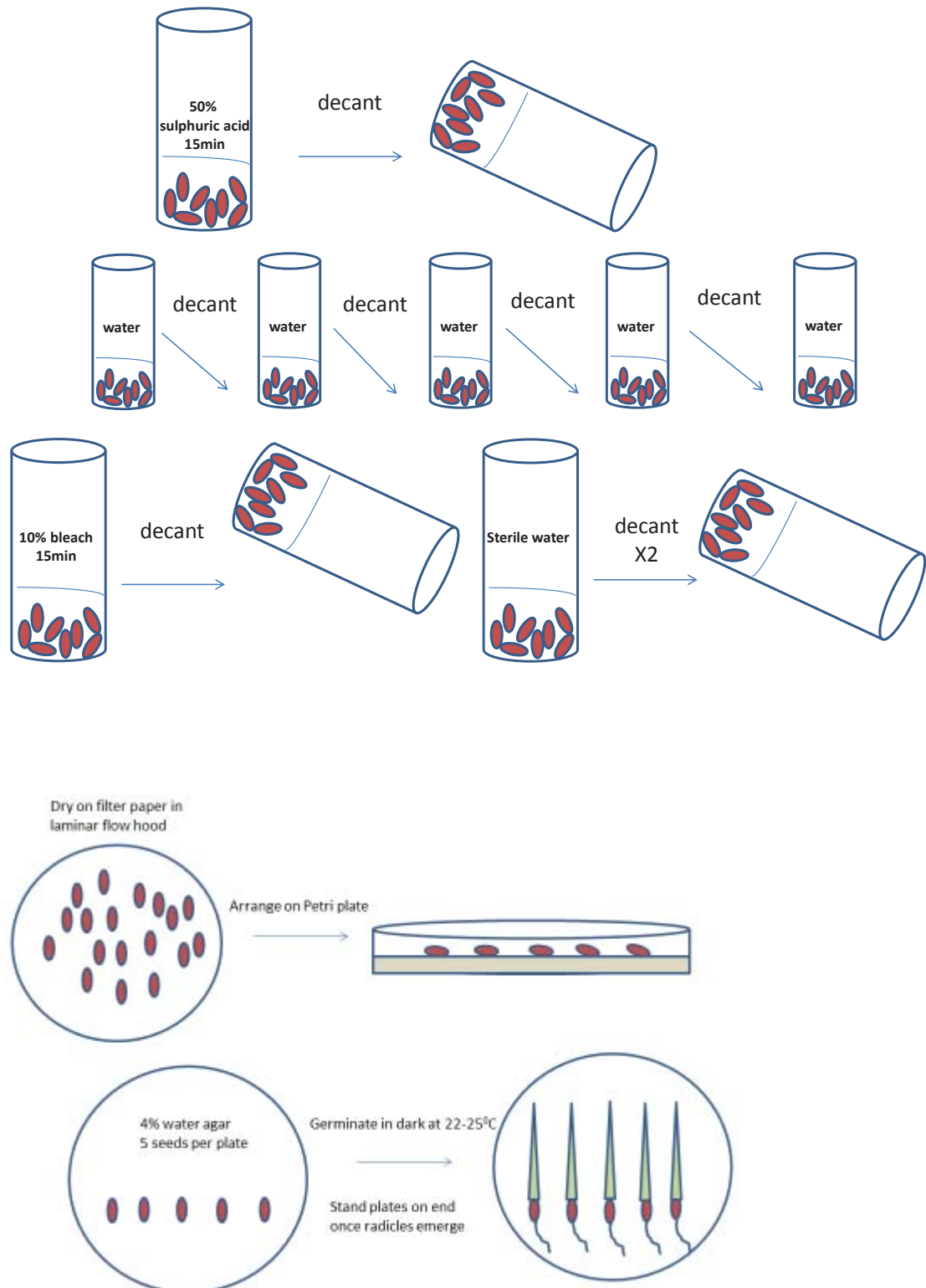
Figure 2.1 Surface sterilisation of seed. A graphical representation of the steps involved in the surface sterilisation of seed for inoculation. The active ingredient in the bleach is sodium hypochlorite.

2.2 Growth Media

2.2.1 Antibiotic ABPDA

Solid media were made using proprietary Potato Dextrose Agar (Difco™ Becton, Dickinson and Co. USA) according to manufacturer's instructions. 19.5g of powder was suspended in 500ml reverse osmosis (RO) water in a 1L Schott bottle and autoclaved at 121°C for 15min. Melted agar was cooled and poured into 9cm diameter sterile plastic Petri plates in a laminar flow cabinet. Just prior to pouring a filter sterilised tetracycline suspension was added to give a final concentration of 5µg/ml.

2.2.2 4% water agar

Water agar (WA) was made by combining 24g of standard agar (Coast Biologicals Ltd, Auckland, NZ) with 600mL RO water in a 1L Shott bottle and autoclaving for 15min at 121°C. Melted agar was cooled and poured into 9cm diameter sterile plastic Petri plates in a laminar flow cabinet.

Figure 2.2 Inoculation of wheat (*Triticum aestivum*) with *Epichloë*. Surface sterilised and etiolated 7-day old 'Monad' wheat seedlings on 4% water agar

2.3   Endophyte detection

2.3.1   Epidermal leaf peel

Tillers were selected from mature plants for endophyte detection. Any necrotic sheath tissue was peeled back off the pseudostem exposing clean, live sheath tissue. The outermost of the remaining sheaths was removed and manipulated under a Zeiss Stemi DRC dissecting microscope at 16x magnification. The sheath was laid on a cutting surface with the adaxial epidermis facing up, a shallow transverse cut was made with a scalpel and #11 blade and the epidermis gently lifted, separated and pulled off the sheath. The epidermal tissue was mounted in a drop of aniline blue stain (glycerol 50 %, lactic acid 25 %, water 24.95 %, aniline blue 0.05 %) on a 25 x 75 x 1 mm microscope slide and covered with a 22 x 22 mm coverslip, heated over a naked flame, allowed to cool and examined at 100x and 400x using a Zeiss compound microscope. Infected plants displayed typical *Epichloë* hyphae growing largely unbranched, longitudinal to the leaf axis (Fig. 2.3).
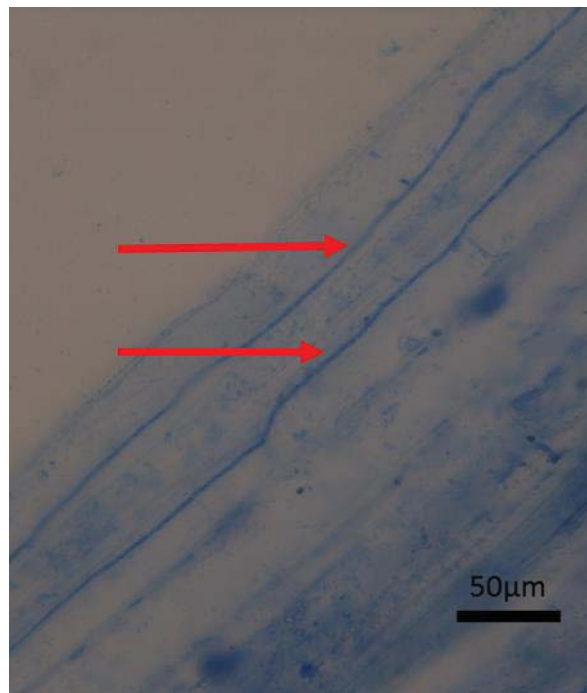
Figure 2.3 Aniline blue stained *Epichloë* in leaf tissue.Hyphae of *Epichloë* strain AR3002-type (red arrows) growing between epidermal cells of 'Rahu' rye (*Secale cereale*) leaf sheath (400x mag)

2.3.2   Seed squash

Grain of wild Hordeeae (*Elymus* and *Hordeum*) or rye, wheat and barley were covered with a 5% sodium hydroxide solution in a heat-proof glass vessel overnight. The following day the solution was decanted and the samples thoroughly rinsed with tap water. Samples were then covered with Garner's solution (0.325g analine blue, 100mL water and 50mL 85% lactic acid) and heated to boiling on a hot plate. After cooling, the palea and lemma were removed and the softened grain mounted on a microscope slide, a cover slip placed over the mounted grain and gentle, even pressure applied squashing the preparation. The preparations were then examined under a compound light microscope at 100x and 400x magnification. Infected grain showed typical even width, serpentine hyphae stained blue by the aniline dye (Fig. 2.4).
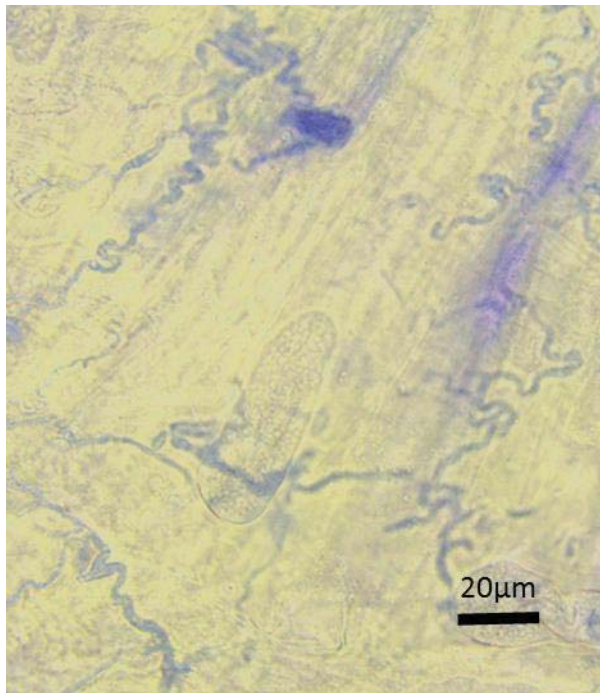
Figure 2.4 Aniline blue stained *Epichloë* in seed. Hyphae of *Epichloë* endophyte growing in seed of 'Monad' wheat (*Triticum aestivum*) 400x mag.

2.3.3   Immuno-detection

Immuno-detection was carried out as described in (Simpson*, et al.*, 2012b). Plants were grown to at least the 3-4 tiller stage before detection of endophyte was undertaken.

Selected tillers were cut basally ca. 5 mm from soil level using a scalpel and #11 blade. Where necrotic sheath tissue was present it was carefully peeled off the tiller before a transverse cut was made on a Perspex cutting board. The freshly cut end of the tiller was gently placed onto a nitrocellulose membrane (NCM) (0.45μm) leaving a circular outline of the moist cut end. Tiller blots were arranged on the NCM in a pattern allowing correct identification of the plant source of each blot. A positive and a negative control tiller were blotted to the membrane using plants of known endophyte status. Blotted membranes were ready for processing immediately but could be retained for at least three weeks, ideally at 4°C, or at ambient conditions prior to processing (Wheatley & Simpson, 2000). Processing: Surfaces on blotted sheets with no bound protein were blocked by immersion in a milk protein blocking solution (BS) (Tris (hydroxymethyl) methylamine 2.42 g, NaCl 2.92 g, Non-fat milk powder 5 g, 1 M HCl 10 ml made up to 1 L with RO water adjusted to pH 7.5) in a 140 x140 mm (600 ml) plastic container. Membranes were shaken on a Bellco mini-orbital shaker (Bellco Biotechnology, Vineland, New Jersey, USA) for at least 2 hours at room temperature. BS was decanted off the membrane and it was rinsed twice with fresh BS before adding 25 μl primary antibody (rabbit anti-endophyte produced at AgResearch in conjunction with Massey University's Small Animal Production unit) in 25 ml BS (1:1000 dilution). Following 15 min shaking at room temperature the membrane was incubated overnight at 4°C. Excess primary antibody was removed by decanting and rinsing twice in fresh BS. The secondary antibody (goat anti-rabbit IgG-AP, sc-2034, Santa Cruz Biotechnology, USA) was added, 6.25 μl in 25 ml BS (1:4,000 dilution) and shaken for 15 min at room temperature before incubating at 4°C for 5 h. Excess secondary antibody was removed by decanting and rinsing twice in BS. Chromogens were prepared by dissolving separately 20mg Fast Red TR (Sigma F-2768) in 12.5 ml Tris buffer (Tris (hydroxymethyl) methylamine 24.2 g in 1 L RO water

adjusted to pH 8.2) and 12.5 mg of napthol AS-MX phosphate (Sigma N4875) in 12.5 ml

Tris buffer per 10 cm^2 of NCM. Chromogen solutions were combined and the NCM

immersed, shaken at room temperature for ca.15 min until red colour develops on control

positive blot (Fig. 2.5). Development was stopped by rinsing three times in RO water.
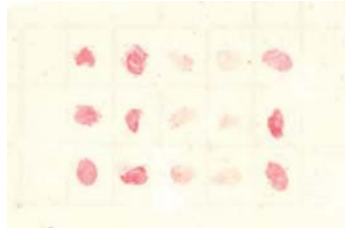
Figure 2.5 Immunoblot of rye (*Secale cereale*) seedlings. Red chromogen-bound tiller imprints from endophyte-infected rye plants. The imprints are made with the cut ends of psuedostems. Image shows *Epichloë* -infected tillers (2 left columns and right column) and endophyte-free tillers (2 middle columns). 2x.

2.4　Simple Sequence Repeats (SSRs), alkaloid determination and beta-tubulin analysis

SSRs were performed by AgResearch (Grasslands) Limited, following the procedure

outlined in Card *et al* (Card*, et al.*, 2014).

Analyses for alkaloid determination were performed at AgResearch using published

methods (Rasmussen *et al.*, 2012; Moore *et al.*, 2015).

Beta-tubulin analysis was performed by AgResearch following methods used in Moon *et al* (Moon*, et al.*, 2004).

2.5　Plant breeding through recurrent selection

Rye plants were selected according to two criteria: infection with *Epichloë* and

morphological phenotype. Following primary infection resulting from inoculation, seed

was harvested from infected plants. Seeds were sown in family (half sibling) sets and

progeny plants examined for the presence of *Epichloë* by immuno-blot (Methods 2.3.3).

Infected plants were visually assessed for stature and general appearance including tiller

number and height. Plants with desirable phenotypes were selected from a number of

families, isolated together and cross-pollinated. Seed was harvested from each mother plant individually, sown, and the selection process repeated for up to four interations.

2.6   Harvest index measurement

Harvest index measurements were taken by severing individual plants at soil level. Grain was manually separated from the plant using a ribbed rubbing board and all chaff was retained. As grain was required for developing the germplasm, a portion (10%) was removed for oven drying along with the non-grain biomass. The harvest index was calculated by dividing the adjusted weight of the dried grain by the combined dried weight of the grain and the cha

Chapter 3

3 Screening wild populations of Hordeeae grasses for the presence of *Epichloë* fungal endophytes

3.1 Introduction

Endophytes identified in the screening section of this project provide the material for inoculation studies and subsequent host breeding and selection. For the purpose of commencing inoculation studies concurrent with the endophyte discovery efforts described below, the *Epichloë* strains of an existing collection were deployed (Appendix 2). The screening was performed with an emphasis on *Elymus* and *Hordeum* species. A focus was to identify plants hosting strains that do not produce the mammalian toxins lolitrem B and ergovaline but that do produce lolines and peramine, which have been shown to confer insect pest deterrence or resistance to infected plants.

The results presented here represent a screen of germplasm held at the Margot Forde Germplasm Centre (MFGC - http://www.agresearch.co.nz/news/margot-forde-germplasm-centre/) consisting of seed accessions sourced from other seed repositories (*ex situ*) (section 3.2) along with a collection made directly from the field at a number of sites in Gansu Province, China (*in situ*) (section 3.3).

3.2 *Ex situ* germplasm screening

215 grass accessions held in the MFGC were screened for *Epichloë* (Appendix 3). These seeds were imported into the MFGC from other germplasm centres; primarily the USDA centre, Pullman, Washington, USA and the Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Germany. They were maintained under low temperature and low relative humidity conditions within the germplasm centre. The MFGC accession prefix

assigned to *Elymus*, *Hordeum* and other species examined in this study, BZ, designates 'other grasses'. The selection for screening was based on geography and/or host species. For the majority of accessions 40 seeds were sown (2 accessions had more: n=50 and 2 had less: n=22 and 34). Germinated seedlings were grown in the glasshouse for 6+ weeks before being examined for *Epichloë* infection using the immuno-blot technique (Methods 2.3.3).

Epichloë was detected in 27 of the 215 accessions using the immuno-blot technique. The infection rate ($\frac{infected\ seedlings}{number\ of\ seed\ sown}$ x 100) ranged from 2.5 to 60%.

Twelve of the 47 species examined (*E. caninus*, *E. elymoides* subsp. *brevifolius*, *E. fedtschenkoi*, *E. glaucissimus*, *E. interruptus*, *E. macrochaetus*, *E. mutabilis*, *E. mutabilis* var. *oschensis*, *E. nevskii*, *E. sibiricus*, *E. trachycaulus* subsp. *subsecundus* and *E. uralensis*) showed evidence of *Epichloë* colonisation (Table 3.1). All of the infected accessions were *Elymus* species.

Table 3.1 Species breakdown of grasses examined for the presence of *Epichloë*. 39 of the 47 species examined were *Elymus* species.* denotes infected accessions

Genus	Species	accessions	infected
Agropyron	*magellanicum*	1	0
Elymus	*abolinii*	1	0
Elymus	*agropyroides*	1	0
Elymus	*andinus*	2	0
Elymus	*antarcticus*	3	0
Elymus	*canadensis*	4	0
Elymus	*caninus**	2	1
Elymus	*dahuricus*	18	0
Elymus	*elymoides ssp. brevifolius**	24	5
Elymus	*elymoides ssp. elymoides*	14	0

Elymus	*fedtschenkoi**	2	1
Elymus	*fibrosus*	2	0
Elymus	*gayanus*	3	0
Elymus	*glaucissimus**	1	1
Elymus	*glaucus*	1	0
Elymus	*interruptus**	2	1
Elymus	*lanceolatus*	2	0
Elymus	*lanceolatus ssp. psammophilus*	1	0
Elymus	*macrochaetus**	3	1
Elymus	*macrourus*	1	0
Elymus	*mutabilis**	25	9
Elymus	*mutabilis ssp. mutabilis*	2	0
Elymus	*mutabilis ssp. praecaespitosus*	6	0
Elymus	*mutabilis var. oschensis**	4	1
Elymus	*nevskii**	19	4
Elymus	*patagonicus*	3	0
Elymus	*pubiflorus*	1	0
Elymus	*repens ssp. elongatiformis*	1	0
Elymus	*repens ssp. repens*	11	0
Elymus	*scabrifolius*	3	0
Elymus	*scabriglumis*	1	0
Elymus	*sibiricus**	17	1
Elymus	*sp.*	2	0
Elymus	*tilcarensis*	1	0
Elymus	*trachycaulus*	9	0
Elymus	*trachycaulus ssp. subsecundus**	1	1
Elymus	*tschimganicus*	3	0
Elymus	*uralensis**	1	1
Elymus	*vaillantianus*	1	0
Elymus	*wiegandii*	1	0

Elytrigia	*intermedia*	1	0
Eremopyrum	*triticeum*	1	0
Hordelymus	*europaeus*	7	0
Hordeum	*bogdanii*	2	0
Hordeum	*brevisubulatum ssp. violaceum*	1	0
Kengyilia	*alatavica*	1	0
Kengyilia	*batalinii*	2	0

Accessions from 17 countries were examined. Infected plants were identified in germplasm originating from 6 countries: Canada, China, Estonia, Kazakhstan, the United States of America and the Russian Federation (Table 3.2). These countries were the most highly represented with regard to the number of accessions examined (n=23, 10, 4, 84, 28 and 34 respectively). China had the highest rate of infected accessions with 3 of the 10 accessions examined being *Epichloë* infected. The USA had the lowest rate of infected accessions with only 1 of 34 examined being infected. The countries where no infected accessions were identified were poorly represented with regard to the number of accessions examined. All of the countries where no infected accession was identified, were represented by ≤ 2 accessions, except for Argentina (n=19).

Table 3.2 Countries of origin of germplasm screened for *Epichloë* infection. * denotes *Epichloë* infection identified

Origin	accessions	infected
Argentina	19	0
Austria	1	0
Belgium	1	0
Canada*	23	6
China*	10	3
Denmark	1	0
Eastonia*	4	1
France	1	0
Germany	1	0
Kazakhstan*	84	9
Kyrgyzstan	2	0
Mexico	1	0
Mongolia	1	0
Pakistan	1	0
Poland	1	0
Russian Fed*	28	7
USA*	34	1

Microsatellites or Simple Sequence Repeats (SSRs) are distributed abundantly in the genome and are highly polymorphic, enabling examination of genetic variation across the *Epichloë* genome and a high resolution discrimination of strains within species. The *Epichloë*-infected accessions identified in the *ex situ* germplasm screen were sampled for SSR analysis. The extraction of DNA that is a prerequisite for the processing and analysis can be performed directly on infected tissues, there is no requirement to isolate the fungus from the plant. A total of 180 plants from 49 accessions were individually

examined and 8 polymorphic SSR loci were probed in the screen. Amongst the 180 plants tested, 32 putatively unique endophyte variants were identified (Table 3.3). These have been clustered into sub-groups based on genetic similarity (Figure 3.1). Fourteen accessions, BZ5591, BZ6464, BZ6955, BZ6989, BZ6993, BZ6994, BZ7008, BZ7291, BZ7292, BZ7293, BZ7503, BZ7808, BZ8452 and BZ8555, yielded more than one endophyte variant.

Table 3.3 Variant groups of *Epichloë* hosted by *Elymus* species

Variant group	Accession_plant No.	Host species	Geography
1	BZ7291_1, 2, 4, 5	*E. mutablilis*	Kazakhstan
2	BZ6989_1, 2, 4	*Elymus sp.*	Tajikistan (site 33)
3	BZ5592_1, 2	*E. nevskii*	Russian Federation
4	BZ7008_1	*Elymus sp.*	Tajikistan (site 31)
5	BZ8561_1 to 6	*E. uralensis*	Kazakhstan
	BZ7301_1	*E. mutablilis*	Russian Federation
	BZ7305_1 to 5	*E. mutablilis*	Russian Federation
	BZ7309_1	*E. mutablilis*	Russian Federation
	BZ7310_1 to 6	*E. mutablilis*	Russian Federation
	BZ5589_1 to 3	*E. mutabilis* var. *oschensis*	Estonia
	BZ7291_3	*E. mutablilis*	Kazakhstan
	BZ7293_1 to 5	*E. mutablilis*	Kazakhstan
6	BZ7292_6	*E. mutablilis*	Kazakhstan
7	BZ5584_1	*E. interruptus*	Canada
8	BZ8555_1 to 3	*E. trachycaulus* ssp. *subsecundus*	Canada
9	BZ8555_4	*E. trachycaulus* ssp. *subsecundus*	Canada
10	BZ6464_1B, 2C	*E. confusus*	Mongolia
11	BZ6464_2A	*E. confusus*	Mongolia
12	PI237707_2 to 6	*Festuca pratensis*	Germany
13	BZ7503_4	*E. sibricus*	Russian Federation
	BZ7808_2, 3	*E. sibricus*	China
	BZ10065	*E. nutans*	China
14	BZ7808_1	*E. sibricus*	China
	BZ10059	*E. dahuricus*	China
	BZ10062	*E. sibricus*	China
15	BZ5578_3	*E. elymoides* ssp. *brevifolius*	Canada
16	BZ7773_1 to 6	*E. elymoides* ssp. *brevifolius*	Canada
	BZ7774_4	*E. elymoides* ssp. *brevifolius*	Canada
	BZ7775_1 to 4	*E. elymoides ssp. brevifolius*	Canada
17	BZ10036_1	*E. dahuricus*	China
	BZ10054	*Elymus sp.*	China
	BZ10060	*E. dahuricus*	China

18	BZ6970_1 to 6	*Hordeum turkestanicum*	Tajikistan (site 19)
19	BZ7291_1A	*E. mutablilis*	Kazakhstan
	BZ7293_6	*E. mutablilis*	Kazakhstan
	BZ6439_1 to 6	*E. caninus*	Kazakhstan
20	BZ6994_5, 6	*Elymus sp.*	Tajikistan (site 34)
	BZ7005_1 to 6	*Elymus sp.*	Tajikistan (site 10)
	BZ6953_1, 2	*Elymus sp.*	Tajikistan (site 10)
	BZ6955_2 to 6	*Elymus sp.*	Tajikistan (site 11)
	BZ6960_1 to 6	*Elymus sp.*	Tajikistan (site 13)
	BZ6961_1, 2	*Elymus sp.*	Tajikistan (site 13)
	BZ6986_1	*Elymus sp.*	Tajikistan (site 30)
	BZ6989_3	*Elymus sp.*	Tajikistan (site 33)
	BZ6989_5, 6	*Elymus sp.*	Tajikistan (site 33)
	BZ10040_1	*E. dahuricus*	China
	BZ10055	*E. dahuricus*	China
	BZ10068	*Elymus sp.*	China
	BZ6994_3	*Elymus sp.*	Tajikistan (site 34)
21	BZ7426_1 to 6	*E. sibricus*	Mongolia
22	BZ6993_1, 4, 5, 6	*Elymus sp.*	Tajikistan (site 34)
	BZ6994_2, 3	*Elymus sp.*	Tajikistan (site 34)
23	BZ10056	*E. dahuricus*	China
24	BZ5591_5	*E. nevskii*	China
	BZ8452_1, 4, 6	*E. nevskii*	China
25	BZ7295_1	*E. mutablilis*	China
	BZ5591_1, 2, 3, 6	*E. nevskii*	China
	BZ7292_2D	*E. mutablilis*	Kazakhstan
	BZ8452_5	*E. nevskii*	China
	BZ6995_1 to 6	*Elymus sp.*	Tajikistan (site 39)
	BZ7008_1	*Elymus sp.*	Tajikistan (site 31)
	BZ6955_1	*Elymus sp.*	Tajikistan (site 11)
	BZ6993_3	*Elymus sp.*	Tajikistan (site 34)
	BZ6994_1	*Elymus sp.*	Tajikistan (site 34)
26	BZ6993_2	*Elymus sp.*	Tajikistan (site 34)
27	BZ7292_1A, 4C, 5B, 13	*E. mutablilis*	Kazakhstan
28	BZ8555_5, 6	*E. trachycaulus* ssp. *subsecundus*	Canada
29	BZ8452_2	*E. nevskii*	China
30	BZ5591_4	*E. nevskii*	China
	BZ8452_3	*E. nevskii*	China
31	BZ7503_1, 2, 3, 5, 6	*E. sibricus*	Russian Federation
32	BZ10052	*E. sibricus*	China
	BZ10067	*E. nutans*	China

Plants hosting representatives of some of the strains were retained and assigned strain numbers. These plants were examine by HPLC and mass spectrometry to determine alkaloid chemistry (Table 3.4).

Table 3.4 Chemistry of representative *Epichloë* variants. √ indicates the presence of the compound in *Epichloë* -infected plant tissue. Ergot alkaloids: (EAs) chanoclavine and ergovaline; Indole diterpenes (IDTs): paspalines, paxillines, terpendoles, lolitrem B and epoxy-janthitrem, peramine and lolines,.including the biosynthetic precursor *exo*-1-acetamidopyrrolizidine (AcAP).

Accession	Original host	Geographic Origin	Peramine	Chanoclavine	Ergovaline	Paspaline Group	Paxilline Group	Terpendole I	Terpendole C	Lolitrem B	Epoxy-Janthitrem I	Lolines
BZ5578	*Elymus elymoides ssp. brevifolius*	Canada	√	√								√
BZ5589	*Elymus mutabilis var. oschensis*	Estonia	√									√
BZ5592	*Elymus nevskii*	Russia	√									√
BZ5591	*Elymus nevskii*	China	√	√	√							
BZ5591	*Elymus nevskii*	China	√	√	√	√						
BZ5578	*E. elymoides ssp. brevifolius*	Canada	√	√								AcAP
BZ7291	*E. mutablilis*	Kazakhstan	√									√
BZ7292	*E. mutablilis*	Kazakhstan	√									√
BZ7310	*E. mutablilis*	Russian Fed	√									√
BZ8555	*E. trachycaulus ssp. subsecundus*	Canada										

1

2 **Figure 3.1 SSR dendrogram. SSR dendrogram made using data from 8 markers showing 32 variants identified in screening of *ex situ* germplasm.**

3 **SSR clades show differences in chemistry shown in blue boxes. Peramine (per), Chanoclavine (chano), Ergovaline (Ev), Indole diterpenes (IDTs),**

4 **Lolines (lol) and *exo*-1-acetamidopyrrolizidine (AcAP).**

3.3   Closest *Epichloë* progentior and hybrid status of strains

An examination was made of an existing collection of *Epichloë* strains using beta tubulin gene analysis. The β-tubulin gene (*tub*2) is a conserved fungal gene that has a varying intronic region (Schardl *et al.*, 1994). Polymorphism of this intronic component of in *Epichloë* populations, (*tub*B), allows the analysis of newly discovered strains and their placement within the context of described *Epichloë* species (Tsai *et al.*, 1994; Moon *et al.*, 2000). The hybrid status can be determined and the closest *Epichloë* progenitor/s can be inferred using this analysis (Moon*, et al.*, 2004).

Strains from Asia, predominantly China, and the USA were examined. The Chinese *Epichloë* populations were dominated by *E. bromicola* and hybrids of *E. bromicola* involving *E. amarillans* and *E. typhina*. The USA strains consisted of *E. elymi* and a hybrid between *E. elymi* and *E. amarillans* (Table 3.5). The *E. bromicola* strains fell into two broad sub-clades that were nominated *E. bromicola* A and *E. bromicola* B. The *E. bromicola* alleles of hybrids all fell within the *E. bromicola* B clade. No *E. bromicola* A hybrids were identified.

Table 3.5 Closest progenitor and hybrid status of *Epichloë* strains. Chinese *Epichloë* consist of *E. bromicola* and its hybrids and *E. yangzii*. The Chinese *Epichloë* hybrids identified showed alleles from a sub-clade of the *E.bromicola* complex, *E. bromicola* B, only. The USA strains consist of *E. elymi* and its hybrid with *E. amarillans*.

AR3004	BZ2157	*Elymus dahuricus*	China	*E. bromicola* A		Non hybrid
AR3010	BZ2198	*Elymus dahuricus*	China	*E. bromicola* A		Non hybrid
AR3016	BZ2162	*Elymus dahuricus*	China	*E. bromicola* A		Non hybrid
AR3022	BZ2162	*Elymus dahuricus*	China	*E. bromicola* A		Non hybrid
AR3043	BZ2162	*Elymus dahuricus*	China	*E. bromicola* A		Non hybrid
AR3044	BZ2162	*Elymus dahuricus*	China	*E. bromicola* A		Non hybrid
AR3058	BZ4948	*Elymus gmelinii*	China	*E. bromicola* A		Non-hybrid
AR3003	BZ2156	*Elymus* sp.	China	*E. bromicola* A		Non-hybrid
AR3006	BZ2160	*Elymus* sp.	China	*E. bromicola* A		Non-hybrid
AR3009	BZ2191	*Hordeum bogdanii*	China	*E. bromicola* A		Non-hybrid
AR3012	PI314696	*Hordeum bogdanii*	China	*E. bromicola* A		Non-hybrid
AR3026	BZ4455	*Hordeum bogdanii*	China	*E. bromicola* A		Non-hybrid
AR3027	BZ4455	*Hordeum bogdanii*	China	*E. bromicola* A		Non-hybrid
AR3028	BZ4455	*Hordeum bogdanii*	China	*E. bromicola* A		Non-hybrid
AR3031	BZ4455	*Hordeum bogdanii*	China	*E. bromicola* A		Non-hybrid
AR3033	BZ4455	*Hordeum bogdanii*	China	*E. bromicola* A		Non-hybrid
AR3034	BZ4455	*Hordeum bogdanii*	China	*E. bromicola* A		Non-hybrid
AR3063	BZ4968	*Hordeum bogdanii*	China	*E. bromicola* A		Non-hybrid
AR3062	BZ5030	*Hordeum roshevitzi*	Russia	*E. bromicola* A		Non-hybrid
AR3056	BZ4944	*Elymus ciliaris*	Russia	*E. bromicola* B		Non-hybrid
AR3065	BZ4897	*Elymus ciliaris*	China	*E. bromicola* B		Non-hybrid
AR3002	BZ2155	*Elymus dahuricus*	China	*E. bromicola* B		Non-hybrid
AR3007	BZ2162	*Elymus dahuricus*	China	*E. bromicola* B		Non-hybrid
AR3023	BZ2162	*Elymus dahuricus*	China	*E. bromicola* B		Non-hybrid
AR3042	BZ2162	*Elymus dahuricus*	China	*E. bromicola* B		Non-hybrid
AR3060	BZ4874	*Elymus dahuricus* subsp. *excelsus*	China	*E. bromicola* B		Non-hybrid
AR3005	BZ2159	*Elymus* sp.	China	*E. bromicola* B		Non-hybrid
AR3020	BZ2160	*Elymus* sp.	China	*E. bromicola* B		Non-hybrid
AR3039	BZ2679	*Elymus caninus*		*E. bromicola* B	*E. amarillans*	Hybrid
AR3046	BZ4833	*Elymus mutabilis*	Kyrgyzstan	*E. bromicola* B	*E. amarillans*	Hybrid
AR3048	BZ4833	*Elymus mutabilis*	Kyrgyzstan	*E. bromicola* B	*E. amarillans*	Hybrid
AR3064	BZ4952	*Elymus mutabilis*	Russia	*E. bromicola* B	*E. amarillans*	Hybrid
AR3014	PI440414	*Hordeum bogdanii*	Kazakhstan	*E. bromicola* B	*E. typhina*	Hybrid
AR3029	BZ4455	*Hordeum bogdanii*	China	*E. bromicola* B	*E. typhina*	Hybrid
AR3061	BZ4969	*Hordeum bogdanii*	China	*E. bromicola* B	*E. typhina*	Hybrid
AR3051	BZ4820	*Elymus virginicus*	USA	*E. elymi*		Non-hybrid
AR3052	BZ4820	*Elymus virginicus*	USA	*E. elymi*		Non-hybrid
AR3054	BZ4820	*Elymus virginicus*	USA	*E. elymi*		Non-hybrid
AR3055	BZ4820	*Elymus virginicus*	USA	*E. elymi*		Non-hybrid
AR3059	BZ4815	*Elymus canadensis*	USA	*E. elymi*	*E. amarillans*	Hybrid
AR3018	BZ2155	*Elymus dahuricus*	China	*E. yangzii*		Non-hybrid
AR3025	BZ 2153	*Elymus dahuricus*	China	*E. yangzii*		Non-hybrid
AR3066	BZ4901	*Elymus dahuricus*	China	*E. yangzii*		Non-hybrid

Determination of the closest *Epichloë* progenitor aids in placing unidentified strains within the context of known characteristics and known hosts of the fungus. SSRs provide a more discriminatory genetic test within the framework that β-tubulin analysis provides,

there is strong concordance of β-tubulin genotypes across SSR clades (Simpson *et al.*, 2012a).

3.4   Collection and screening of *in situ Elymus* germplasm

A field collection was made with the express purpose of securing germplasm for this project. A three day collection was made in Gansu province, North West China during August 2012 (Fig. 3.2). The collection trip started at Lanzhou, south Gansu, and involved a journey of over 600 kms along the Hexi corridor. There were 17 collection sites at five locations (Fig. 3.3) at elevations from 1537m – 2770m. Close to 500 *Elymus* accessions were collected. Each accession represented seed progeny of an individual plant.

Seed of 202 accessions from Sandan (n=2), Minle (n=29), Sunan (n=108), Tianzhu (n=25) and Yongdeng (n=15) were sown at Palmerston North and plants examined for *Epichloë* infection using the immuno-blot technique (Table 3.6). Ninety nine (99) infected accessions were identified comprised of 20 genetically distinct variants based upon SSR analysis using 25 polymorphic markers (Table 3.7).
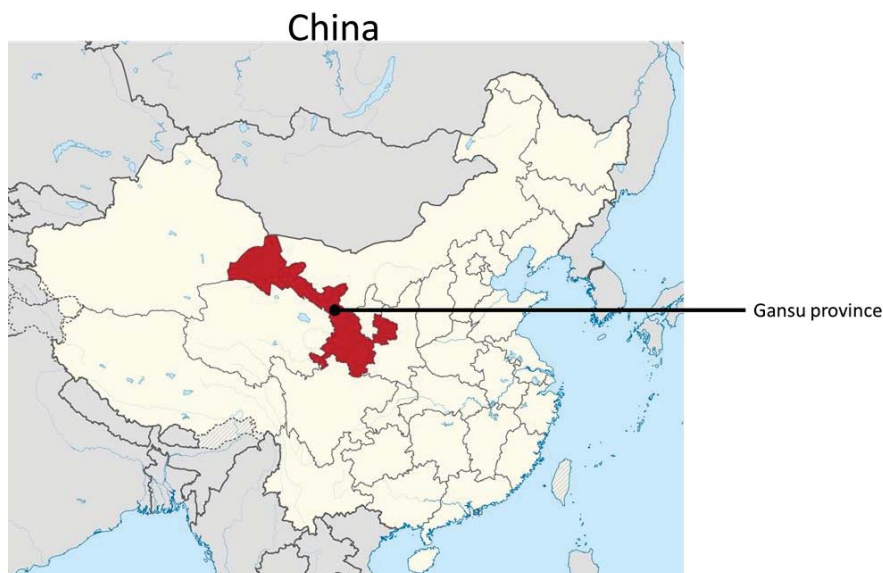
Figure 3.2 Map of China. Showing where *in situ Elymus* germplasm collection was made. Gansu province is shown in red.

Gansu province
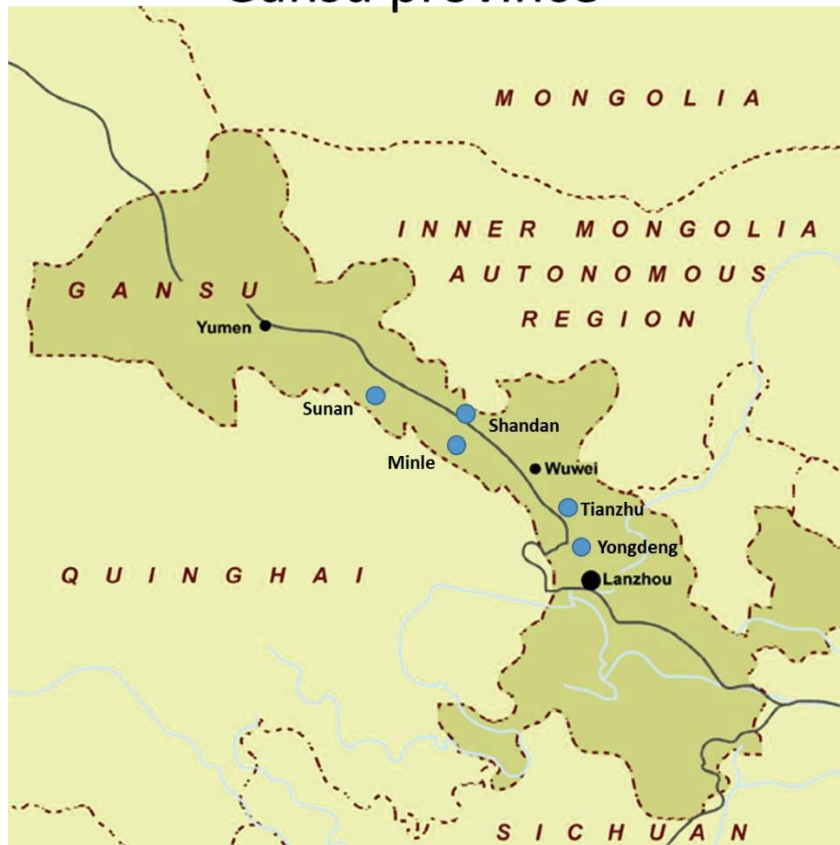
Figure 3.3 Map of Gansu province. Detail of Gansu province showing collection regions (blue dots) from where the *in situ Elymus* germplasm was collected.

Table 3.6 Infected accessions of *Elymus* collected in Gansu Province, China in 2012 (n=99)

Accession		Collection details	Genus	Species	Site
BZ	9985	Gansu, China Collection 2012 sly022	*Elymus*	*cylindricus*	site 3
BZ	9988	Gansu, China Collection 2012 sly0226	*Elymus*	*cylindricus*	site 3
BZ	9991	Gansu, China Collection 2012 sly029	*Elymus*	*cylindricus*	site 3
BZ	9986	Gansu, China Collection 2012 sly024	*Elymus*	*dahuricus*	site 3
BZ	9989	Gansu, China Collection 2012 sly027	*Elymus*	*dahuricus*	site 3
BZ	9982	Gansu China Collection 2012 sly018	*Elymus*	sp.	site 3
BZ	9977	Gansu China Collection 2012 sly013	*Elymus*	sp.	site 3
BZ	9978	Gansu China Collection 2012 sly014	*Elymus*	sp.	site 3
BZ	9979	Gansu China Collection 2012 sly015	*Elymus*	sp.	site 3
BZ	9984	Gansu China Collection 2012 sly021	*Elymus*	sp.	site 3
BZ	9976	Gansu China Collection 2012 sly011	*Elymus*	sp.	site 3
BZ	9980	Gansu China Collection 2012 sly016	*Elymus*	sp.	site 3
BZ	9981	Gansu China Collection 2012 sly017	*Elymus*	sp.	site 3
BZ	9992	Gansu, China Collection 2012 sly034	*Elymus*	*dahuricus*	site 4
BZ	9994	Gansu China Collection 2012 sly035	*Elymus*	sp.	site 4

BZ	9995	Gansu China Collection 2012 sly036	*Elymus*	sp.	site 4
BZ	9997	Gansu China Collection 2012 sly038	*Elymus*	sp.	site 4
BZ	9998	Gansu China Collection 2012 sly051	*Elymus*	sp.	site 4
BZ	9999	Gansu China Collection 2012 sly052	*Elymus*	sp.	site 4
BZ	10000	Gansu China Collection 2012 sly054	*Elymus*	sp.	site 4
BZ	10005	Gansu China Collection 2012 sly095	*Elymus*	*dahuricus*	site 6
BZ	10002	Gansu China Collection 2012 sly083	*Elymus*	*dahuricus*	site 6
BZ	10003	Gansu China Collection 2012 sly093	*Elymus*	*dahuricus*	site 6
BZ	10004	Gansu China Collection 2012 sly094	*Elymus*	*dahuricus*	site 6
BZ	10006	Gansu China Collection 2012 sly096	*Elymus*	*dahuricus*	site 6
BZ	10007	Gansu China Collection 2012 sly097	*Elymus*	*dahuricus*	site 6
BZ	10001	Gansu China Collection 2012 sly082	*Elymus*	sp.	site 6
BZ	10021	Gansu China Collection 2012 sly114	*Elymus*	*cylindricus*	site 7
BZ	10015	Gansu China Collection 2012 sly108	*Elymus*	*dahuricus*	site 7
BZ	10016	Gansu China Collection 2012 sly109	*Elymus*	*dahuricus*	site 7
BZ	10022	Gansu China Collection 2012 sly115	*Elymus*	*dahuricus*	site 7
BZ	10023	Gansu China Collection 2012 sly116	*Elymus*	*dahuricus*	site 7
BZ	10029	Gansu China Collection 2012 sly131	*Elymus*	*dahuricus*	site 7
BZ	10017	Gansu China Collection 2012 sly111	*Elymus*	*sibiricus*	site 7
BZ	10018	Gansu China Collection 2012 sly110	*Elymus*	*sibiricus*	site 7
BZ	10019	Gansu China Collection 2012 sly112	*Elymus*	*sibiricus*	site 7
BZ	10020	Gansu China Collection 2012 sly113	*Elymus*	*sibiricus*	site 7
BZ	10025	Gansu China Collection 2012 sly118	*Elymus*	*sibiricus*	site 7
BZ	10011	Gansu China Collection 2012 sly102	*Elymus*	sp.	site 7
BZ	10012	Gansu China Collection 2012 sly104	*Elymus*	sp.	site 7
BZ	10013	Gansu China Collection 2012 sly105	*Elymus*	sp.	site 7
BZ	10030	Gansu China Collection 2012 sly139	*Elymus*	*dahuricus*	site 8
BZ	10039	Gansu China Collection 2012 sly158	*Elymus*	*dahuricus*	site 8
BZ	10032	Gansu China Collection 2012 sly140	*Elymus*	*dahuricus*	site 8
BZ	10033	Gansu China Collection 2012 sly141	*Elymus*	*dahuricus*	site 8
BZ	10034	Gansu China Collection 2012 sly142	*Elymus*	*dahuricus*	site 8
BZ	10035	Gansu China Collection 2012 sly143	*Elymus*	*dahuricus*	site 8
BZ	10031	Gansu China Collection 2012 sly137	*Elymus*	sp.	site 8
BZ	10132	Gansu China Collection 2012 sly270	*Elymus*	*dahuricus*	site 10
BZ	10103	Gansu China Collection 2012 sly241	*Elymus*	*dahuricus*	site 10
BZ	10133	Gansu China Collection 2012 sly271	*Elymus*	*dahuricus*	site 10
BZ	10136	Gansu China Collection 2012 sly274	*Elymus*	*dahuricus*	site 10
BZ	10073	Gansu China Collection 2012 sly211	*Elymus*	*dahuricus*	site 10
BZ	10088	Gansu China Collection 2012 sly226	*Elymus*	*dahuricus*	site 10
BZ	10089	Gansu China Collection 2012 sly227	*Elymus*	*dahuricus*	site 10
BZ	10090	Gansu China Collection 2012 sly228	*Elymus*	*dahuricus*	site 10
BZ	10091	Gansu China Collection 2012 sly229	*Elymus*	*dahuricus*	site 10
BZ	10096	Gansu China Collection 2012 sly234	*Elymus*	*dahuricus*	site 10
BZ	10097	Gansu China Collection 2012 sly235	*Elymus*	*dahuricus*	site 10
BZ	10098	Gansu China Collection 2012 sly236	*Elymus*	*dahuricus*	site 10
BZ	10099	Gansu China Collection 2012 sly237	*Elymus*	*dahuricus*	site 10

BZ	10100	Gansu China Collection 2012 sly238	*Elymus*	*dahuricus*	site 10
BZ	10102	Gansu China Collection 2012 sly240	*Elymus*	*dahuricus*	site 10
BZ	10104	Gansu China Collection 2012 sly242	*Elymus*	*dahuricus*	site 10
BZ	10125	Gansu China Collection 2012 sly263	*Elymus*	*dahuricus*	site 10
BZ	10137	Gansu China Collection 2012 sly276	*Elymus*	*dahuricus*	site 10
BZ	10074	Gansu China Collection 2012 sly212	*Elymus*	*dahuricus*	site 10
BZ	10107	Gansu China Collection 2012 sly245	*Elymus*	*dahuricus*	site 10
BZ	10108	Gansu China Collection 2012 sly246	*Elymus*	*dahuricus*	site 10
BZ	10109	Gansu China Collection 2012 sly247	*Elymus*	*dahuricus*	site 10
BZ	10095	Gansu China Collection 2012 sly233	*Elymus*	*dahuricus*	site 10
BZ	10093	Gansu China Collection 2012 sly231	*Elymus*	*nutans*	site 10
BZ	10117	Gansu China Collection 2012 sly255	*Elymus*	*nutans*	site 10
BZ	10119	Gansu China Collection 2012 sly257	*Elymus*	*nutans*	site 10
BZ	10120	Gansu China Collection 2012 sly258	*Elymus*	*nutans*	site 10
BZ	10124	Gansu China Collection 2012 sly262	*Elymus*	*nutans*	site 10
BZ	10130	Gansu China Collection 2012 sly268	*Elymus*	*nutans*	site 10
BZ	10092	Gansu China Collection 2012 sly230	*Elymus*	*nutans*	site 10
BZ	10118	Gansu China Collection 2012 sly256	*Elymus*	*nutans*	site 10
BZ	10075	Gansu China Collection 2012 sly213	*Elymus*	*nutans*	site 10
BZ	10094	Gansu China Collection 2012 sly232	*Elymus*	*nutans*	site 10
BZ	10114	Gansu China Collection 2012 sly252	*Elymus*	*sibiricus*	site 10
BZ	10081	Gansu China Collection 2012 sly219	*Elymus*	sp.	site 10
BZ	10086	Gansu China Collection 2012 sly224	*Elymus*	sp.	site 10
BZ	10138	Gansu China Collection 2012 sly465	*Elymus*	*dahuricus*	site 15
BZ	10147	Gansu China Collection 2012 sly475	*Elymus*	*sibiricus*	site 15
BZ	10149	Gansu China Collection 2012 sly477	*Elymus*	*sibiricus*	site 15
BZ	10139	Gansu China Collection 2012 sly466	*Elymus*	sp.	site 15
BZ	10155	Gansu China Collection 2012 sly497	*Elymus*	*dahuricus*	site 16
BZ	10169	Gansu China Collection 2012 sly511	*Elymus*	*dahuricus*	site 16
BZ	10156	Gansu China Collection 2012 sly498	*Elymus*	*dahuricus*	site 16
BZ	10158	Gansu China Collection 2012 sly500	*Elymus*	*dahuricus*	site 16
BZ	10159	Gansu China Collection 2012 sly501	*Elymus*	*dahuricus*	site 16
BZ	10161	Gansu China Collection 2012 sly503	*Elymus*	*dahuricus*	site 16
BZ	10162	Gansu China Collection 2012 sly504	*Elymus*	*dahuricus*	site 16
BZ	10166	Gansu China Collection 2012 sly508	*Elymus*	*dahuricus*	site 16
BZ	10167	Gansu China Collection 2012 sly509	*Elymus*	*dahuricus*	site 16
BZ	10168	Gansu China Collection 2012 sly510	*Elymus*	*dahuricus*	site 16
BZ	10157	Gansu China Collection 2012 sly499	*Elymus*	sp.	site 16

Infected accessions were identified at Sandan (site 3 and 4), Minle (site 6 and 7), Sunan (site 8 and 10), Tianzhu (site 15) and Yongdeng (site 16). Between 2 (Yongdeng) and 13 (Sunan) genotypes were identified, using SSR analysis, at the five regions. As the number

of accessions obtained and analysed from each region varied, the genetic density is expressed as a diversity index (Table 3.7).

Table 3.7 Accessions collected, number infected and genotypes identified from five regions of Gansu Province, China. Diversity index (genotypes/infected accessions x 100)

Site	accessions	infected	% infected	genotypes	diversity index
Sandan	25	20	80	4,5,11,14,16,17 (n=6)	0.30
Minle	29	21	72	2,5,6,15 (n=4)	0.19
Sunan	108	43	40	5,6,7,9,10,11,12,13,14,16,18,19,20 (n=13)	0.32
Tianzhu	25	4	16	1,3,8 (n=3)	0.75
Yongdeng	15	11	73	5,16 (n=2)	0.18

At just one site, site 10, one of the collection sites at Sunan (Fig. 3.4), ten genetically distinct strains were identified from 36 infected accessions of four *Elymus* species. One of the identified species, *Elymus dahuricus*, was found to be hosting 7 genetically distinct *Epichloë* strains whereas only one genetically distinct strain was identified in the species *E. sibiricus* and *E. tangutorum* respectively. Whilst most SSR variants were only present in one or two different *Elymus* species, SSR variant 14 was identified in three species; *E. dahuricus*, *E. nutans* and *E. tangutorum* (Table 3.8).

Table 3.8 SSR genotypes identified in germplasm from one site (site 10) of Gansu Province collection

SSR variant	E. dahuricus	E. nutans	E. sibiricus	E. tangutorum
9		√		
10		√	√	
11	√			
12	√			
13	√			
14	√	√		√
16		√		
18	√			
19	√			
20	√			

Site 10   N: 38°   48.849'   E: 99° 35.470'

78 accessions - 36 infected - 10 SSR genotypes

Figure 3.4 Collection site number 10 Sunan, Gansu Province, China. Germplasm was collected on both sides of the road over a 600 m distance. The road ran alongside a small stream to the right of the image.

Site 15 (Fig. 3.5), is notable in that an *Epichloë* strain with an SSR genotype significantly

different from the others in the collection was identified.

Site 15   N: 37°   06.471'   E: 102° 57.524'

50 accessions - 4 infected - 3 SSR genotypes

Figure 3.5 Collection site number 15 Tianzhu, Gansu Province, China. At this site 50 single-plant accessions were collected. Four of these accessions were shown to be infected. SSR analysis showed that three distinct genotypes were present.

3.5 Screening of *Aegilops* germplasm

Aegilops is a Hordeeae genus of 22 species that is notable in that one of the species, *A. tauschii* is the contributor of the D genome to hexaploid wheat. Although in this study *Elymus* and *Hordeum* were the source grasses of the *Epichloë* used in synthetically infecting wheat, an *Epichloë* isolated from an *Aegilops* species would represent a strain from a host more closely related to modern wheat than either *Elymus* or *Hordeum*. Given the host-specificity of *Epichloë*, with implications for the establishment of synthetic symbioses, the closer the *Epichloë* source and destination hosts are genetically then the more likely a successful infection may be. There may also be some value in identifying *Epichloë*-infected *Aegilops* from the perspective of genetic relatedness to the

B genome component of wheat despite an *Aegilops* contribution to the B genome of wheat being contested (Huang, *et al.*, 2002; Kilian *et al.*, 2007; Gornicki *et al.*, 2014). It is likely that the S genome of *Aegilops speltoides* diverged very early from the progenitor of the B genome (Salse *et al.*, 2008). In addition to being a source of fungal culture for inoculation studies, infected *Aegilops* could be used in either the establishment of synthetic wheat by hybridising with a tetraploid *Triticum* or by introgression into hexaploid cultivars (Ogbonnaya *et al.*, 2013; Li *et al.*, 2014; Gul *et al.*, 2015). For example, *Aegilops markgrafii* has been deployed to produce wheat introgression lines with mildew resistance (Knüpffer, 2009).

A screen of *Aegilops* species was performed using germplasm sourced from Greece, Cyprus, Tajikistan, Georgia and Armenia. One hundred and eight (108) accessions were examined representing nine named species; *Aegilops biuncialis* (25 accessions), *A. caudata* (3 accessions), *A. comosa* (6 accessions), *A. cylindrica* (18 accessions), *A. geniculata* (6 accessions), *A. neglecta* (8 accessions), *A. peregrina* (1 accession), *A. tauschii* (7 accessions) and *A. triuncialis* 32 accessions). Twenty four (24) seed of each accession were sown for all except for 6 accessions where fewer than 24 (4-21) seed were available for screening. Plants were grown and examined for the presence of *Epichloë* using the immunoblot assay (Methods 2.3.3). No infected individuals were identified amongst the 2,505 plants examined.

3.6   Screening Hordeeae grasses for *Epichloë*

Screening germplasm for the presence of *Epichloë* is an essential prerequisite to establishing synthetic symbioses in target grasses. Here a range of germplasm was examined; both established collections and a bespoke collection for the purpose of this study. As seed is stored, the viability of *Epichloë* reduces (Hume *et al.*, 2011), this, coupled with the use of fungicides on accession-increase crops and the possible effects

on *Epichloë* infection, make the examination of freshly collected seed a more desirable proposition, despite the additional resource required.

The strains identified here constitute a resource that can be deployed in inoculation studies. Due to time limitations and the need to commence inoculation studies in parallel with endophyte discovery efforts, previously identified strains have been used in the following section.

Chapter 4

4 Synthetic symbioses

4.1 Introduction

Although *Epichloë* endophytes are obligate biotrophs, they can be cultured on artificial media in a saprotrophic state. This cultured form of the fungus can be used as inoculum to establish a synthetic, systemic symbiosis in a new host. This method of isolation of *Epichloë* and synthetic infection is commonly practiced with pasture grasses where the source host and the new host are the same species. For example, strains of the perennial ryegrass (PRG) endophyte *Epichloë festucae* var *lolii* are isolated from wild PRG populations and characterised. Strains that do not produce mammalian toxins are inoculated into cultivated populations of PRG to establish cultivars that are resistant to biotic and abiotic stresses but are not toxic to grazing animals (Johnson*, et al.*, 2013).

In this part of the study a similar approach has been undertaken, utilising *Epichloë* isolated from grasses in the same tribe as the targeted cereal grasses, to establish synthetic symbioses utilising strains screened and characterised as described in Chapter 3. As the target cereal grasses do not naturally host *Epichloë*, the inoculum for these experiments is sourced from the closest relatives that are natural hosts. Both outcrossing (rye) and selfing, (wheat and barley), cereal grasses are used as potential hosts. Addtionally, a rye line used in a hybrid seed production system that is selfing is used in the inoculation experiments.

4.2 Inoculation of rye (*Secale cereale*)

The first example of infection of a Hordeeae cereal was that of a triticale (xTriticosecale - *Triticum* x *Secale*) line DH100 with *Epichloë* strain AR3018 (ex *Elymus dahuricus* − accession BZ2155 collected in China). The phenotype of the infected triticale was poor

and plants died before reaching maturity. However, this result, combined with the inability, at that time, to infect *Triticum*, led to efforts to infect rye (*Secale*). Early inoculations of rye cultivars 'Rahu' and 'Amilo' with strain AR3018 resulted in high numbers of infected plants, but, as with the triticale, the phenotypes were poor and the plants died before reproducing. Further inoculations, with strains beyond AR3018, resulted in the infection of 'Amilo' rye with a number of strains (Table 4.1) including AR3056 (ex *Elymus ciliaris* – accession BZ4944 collected in the Russian Federation). In 2009, three grains were harvested from a single AR3056-infected plant and, from these, a single infected progeny plant was generated. This single infected plant, with a phenotype similar to an un-infected plant (Fig. 4.1), was pollinated by endophyte-free 'Amilo' rye plants and produced 24 grains.
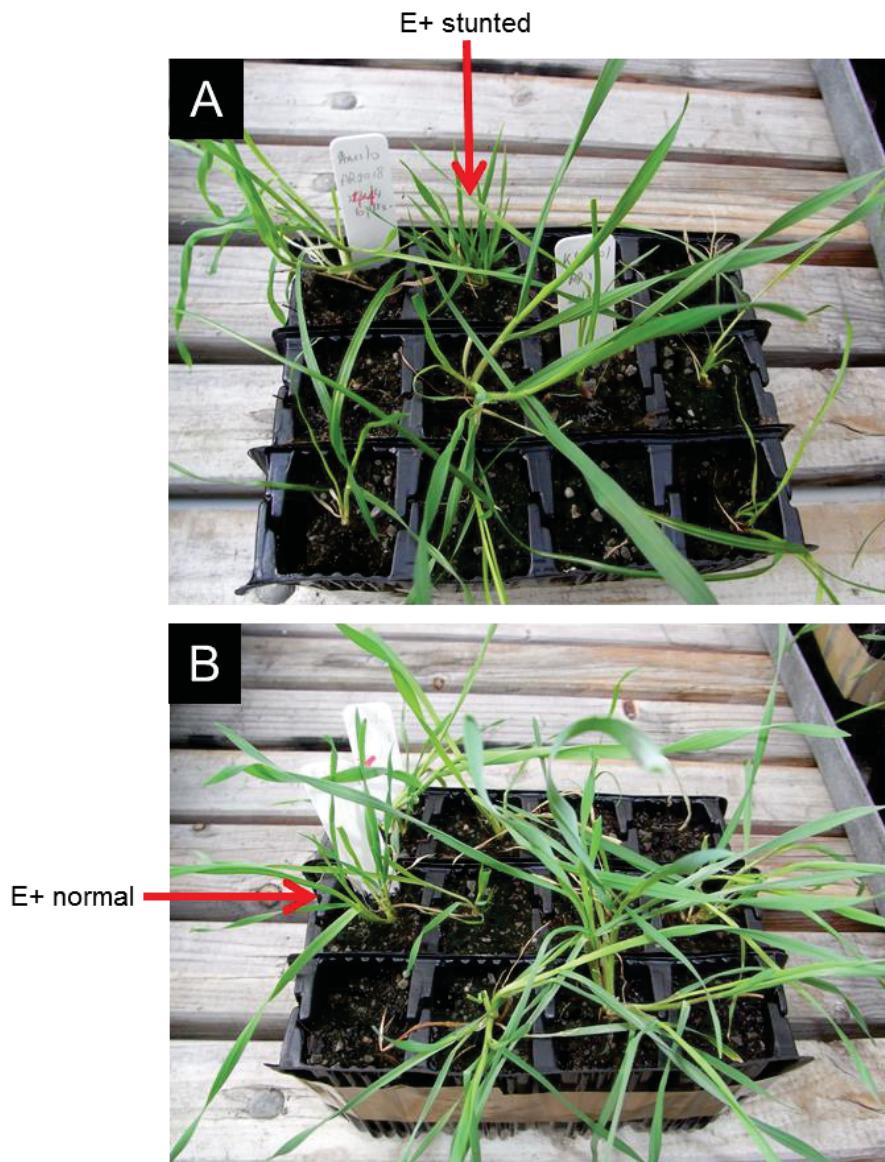
Figure 4.1 Rye infection phenotypes. (A) 'Amilo' rye inoculated with AR3018 an *Epichloë* isolated from *Elymus dahuricus* – red arrow indicating infected plant displaying dwarfed phenotype. (B) 'Amilo' rye inoculated with AR3056 an *Epichloë* isolated from *Elymus ciliaris* – red arrow indicates an infected plant with normal phenotype

Inoculation of 'Rahu' rye was undertaken with both non-hybrid (e.g AR3002, AR3005, AR3007, AR3017) and then hybrid (e.g AR3046, AR3067, AR3068, AR3074, AR3078) strains (Table 4.2). Infections were obtained in 'Rahu' with a range of infection phenotypes resulting (Fig. 4.2).

Table 4.1 'Amilo' rye (*Secale cereale*) inoculation results (as determined by immunoblot) using a range of hybrid (H) and non-hybrid (NH) *Epichloë* strains. ND = not determined.

	Amilo			
Endo strain	inoculated	positive	negative	live % positive
AR3002(NH)	41	7	20	26
AR3005(NH)	40	5	17	23
AR3007(NH)	39	8	17	32
AR3013(ND)	46	4	13	24
AR3014(H)	41	0	27	0
AR3015(NH)	44	5	22	19
AR3017(NH)	43	14	7	67
AR3018(NH)	41	29	4	88
AR3020(NH)	42	12	16	43
AR3023(NH)	42	11	21	34
AR3025(NH)	40	22	5	81
AR3029(H)	42	0	20	0
AR3039(H)	41	11	21	34
AR3042(NH)	41	10	21	32
AR3046(H)	42	17	11	61
AR3056(NH)	44	13	15	46

Table 4.2 'Rahu' rye (*Secale cereale*) inoculation results (as determined by immunoblot) using a range of hybrid (H) and non-hybrid (NH) *Epichloë* strains. ND = not determined.

Endo strain	inoculated	positive	negative	live % positive
			Rahu	
AR3002(NH)	41	6	8	43
AR3005(NH)	41	12	3	80
AR3007(NH)	40	8	8	50
AR3013(ND)	41	12	9	57
AR3015(NH)	42	6	11	35
AR3017(NH)	41	10	5	67
AR3020(NH)	40	7	17	29
AR3023(NH)	39	1	3	25
AR3039(H)	31	10	11	48
AR3042(NH)	41	9	6	60
AR3046(H)	44	18	17	51
AR3056(NH)	40	6	12	33
AR3067(H)	50	9	35	20
AR3068(H)	50	8	33	20
AR3074(H)	50	6	38	14
AR3078(H)	50	3	26	10

Figure 4.2 Infection phenotypes. "Rahu' rye infected with AR3046 an *Epichloë* isolated from *Elymus mutabilis*. (A) a normal phenotype infected plant. All floral tillers were confirmed infected by isolating fungus from the flagleaf. (B) a dwarfed phenotype infected plant – this plant did not flower.

These inoculation studies demonstrate that both a milling rye cultivar ('Amilo') and a forage rye ('Rahu') can be infected with both hybrid and non-hybrid *Epichloë* strains. Survival and infection rates vary, the symbiosis phenotype also varies.

4.3 Synthetically infected rye are colonised to the leaf tip

A feature of *Epichloë* infection of the Poeae grasses *Festuca* and *Lolium* is the paucity or absence of hyphae colonising the upper regions of leaf blade tissue (Christensen*, et al.*, 2002). Isolation of *Epichloë* from surface sterilised excised leaf tissue of infected rye (Methods 2.1.1) in this study has demonstrated that *Epichloë* hyphae colonise the host tissue of these associations all through the leaf blade including the tip (Fig. 4.3). This colonisation pattern was also observed in the wild naturally infected Hordeeae grasses *Elymus* and *Hordeum*.
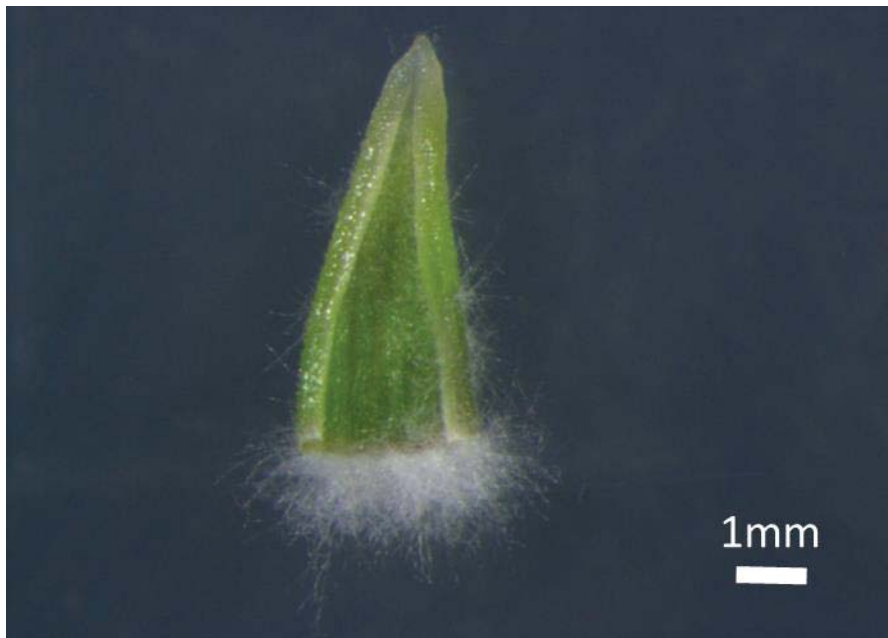
Figure 4.3 *Epichloë* growing from the surface sterilised leaf tip on PDA. AR3002-type emerging from excised 'Rahu' rye tissue.

4.4 Inoculation of selfing rye

Although *Secale cereale* L. is an obligate outcrossing species, a low level of selfing occurs naturally, and exploitation of this has enabled the development of selfing lines. Combining the production of selfing lines with the ability to produce male sterile analogues allows the maintenance of a male sterile homozygous line that, when

hybridised with an unrelated line carrying a fertility restorer, produces a rye cultivar exhibiting hybrid vigour. An experiment was undertaken to examine the effect of infecting a cytoplasmic male sterile (cms) homozygous rye, Lo6-P and its male fertile selfed analogue, Lo6-N. The Lo6 lines were obtained from KWS LOCHOW GMBH. Seedlings were germinated and inoculated (Methods 2.1.2) with an AR3002-type strain. Inoculated seedlings were grown on in potting soil and subsequently examined to determine infection status. Of the 61 inoculated Lo6-N (male fertile) seedlings, 53 (87%) became infected. Similarly, 24 of the 48 (50%) inoculated Lo6-P (cms) seedlings became infected (Table 4.3).

Table 4.3 Inoculation results of orthologous selfing rye lines Lo6-N (male fertile) and Lo6-P (cytoplasmic male sterile) using AR3002-type *Epichloë*

Host	endophyte	inoculated	positive	negative	live % positive
Lo6-N	AR3002-type	61	53	4	93
Lo6-P	AR3002-type	48	24	19	56

4.5 Inoculation of hexaploid *Triticum* with *Epichloë* isolated from hexaploid *Elymus*

As with synthetic infection of rye, the *Epichloë* strains selected as inoculum for wheat (*Triticum aestivum*) were sourced from *Elymus* and tertiary gene-pool *Hordeum*. The ploidy of *Elymus* species varies, the majority being tetraploid, however other ploidys, including hexaploid, occur in nature. Given the hexaploid status of bread wheat, an experiment was performed where *Epichloë* strains isolated from hexaploid *Elymus* were inoculated into 'Monad' wheat. *Epichloe* was isolated from from six accessions, including two *Elymus* species; *E. canadensis* and *E. dahuricus* and one sub-species *E. dahuricus* subsp. *excelsus* (Table 4.4). Between 41 and 45 plants of each accession were inoculated. Only one accession, *E. dahuricus* subsp. *excelsus*, yielded positive plants, with a 12% infection rate.

Table 4.4 *Triticum* inoculations with *Epichloë* from hexaploid *Elymus*

'Monad' wheat (*Triticum aestivum*)				
Epichloë source	inoculated	positive	negative	live % positive
Elymus dahuricus	42	0	18	0
E. dahuricus	43	0	13	0
E. canadensis	41	0	22	0
E. dahuricus subsp. excelsus	45	6	11	35
E. dahuricus	42	0	6	0

4.6 Inoculation of spring and winter wheat

The *E. dahuricus* strain AR3018, that infected rye, was also shown to infect wheat with a similar stunted phenotype resulting. AR3018 along with the *E. dahuricus* subsp. *excelsus* strain AR3060 were used in an experiment examining different potential hosts. The experiment was conducted to examine infection ability and frequencies of the two strains of *Epichloë* into two cultivars of wheat. Strains AR3018 and AR3060 were inoculated into wheat cultivars 'Monad', a spring wheat and 'Savannah', a winter wheat. The *Epichloë* strain AR3018 isolated from *Elymus dahuricus*, collected in China (accession BZ 2155), clusters with *Epichloë yangzii* (using β-tubulin data). AR3060 isolated from *Elymus dahuricus* subspecies *excelsus*, also from China (accession BZ 4874), clusters with *Epichloë bromicola* (Appendix 1). One hundred 'Savannah' seedlings and 100 'Monad' seedlings were inoculated with AR3018 and another 100 of each cultivar were inoculated with AR3060. An additional 300 'Monad' seedlings were inoculated with AR3018 to generate infected vegetative material for chemical analysis. The infection data from these additional seedlings were included in the inoculation results.

After inoculation, the seedlings were grown in soil for several weeks before sampling for infection success. For all inoculation sets, some plants died before the end of this growing

period. The death rates ranged from 13- 22% across the two cultivars, with means of 20%

for 'Monad' and 17% for 'Savannah'. Successful infection was identified in 'Monad'

only. The frequency of infection of the surviving 'Monad' plants was 4% for AR3018

and 31% for AR3060 (Table 4.5).

Table 4.5 Infection of 'Monad' and 'Savannah' wheat following inoculation with strains AR3018 and AR3060

Host	strain	inoculated	positive	negative	dead	live % positive
Monad	AR3018	400	12	299	89	4
Monad	AR3060	100	26	57	17	31
Savannah	AR3018	100	0	79	21	0
Savannah	AR3060	100	0	87	13	0

The morphological phenotypes of the infected 'Monad' wheat plants differed with

endophyte strain. The AR3018-infected plants were stunted, dark green and died before

flowering (Figure 4.2). The AR3060-infected plants were also reduced in stature, but not

as severely as the AR3018-infected plants (Figure 4.3). The AR3060-infected seedlings

developed into plants that flowered and set seed. An examination of seed harvested from

AR3060-infected plants using the seed squash method (Methods 2.3.2) showed that the

fungus had colonised the seed (Chapter 2 Fig 2.4). This result represents the first record

of the systemic infection of a modern hexaploid wheat with *Epichloë* and transmission to

the seed demonstrating a full infection lifecycle.

Figure 4.4 Un-infected and infected wheat Un-infected (left) and AR3018-infected (right) wheat

Figure 4.5 Un-infected and infected wheat. Un-infected (left) and AR3060-infected (right) 'Monad' wheat

4.7 Inoculation of wheat, barley and triticale cultivars with strain AR3060

Because *Epichloë* strain AR3060 could infect 'Monad' wheat giving viable phenotypes and endophyte transmission to the seed, this strain was selected for an inoculation study involving a selection of cultivars of wheat, barley and triticale. This strategy of using a single *Epichloë* strain and a range of potential host genotypes, 'strategy one', was deployed as the first of two strategies, the second strategy to use a limited number of potential hosts identified in 'strategy one' and attempt infection with an extended number of *Epichloë* strains, 'strategy two'. Earlier inoculation studies (Section 4.6) had shown infection frequencies as low as 3% - for this reason the minimum number of seedlings for inoculation efforts was determined as fifty individuals. Attempts were made to generate 50 clean (absence of contaminant fungi and bacteria) viable seedlings for the following study, however this was not always achieved. Up to 50 seedlings (21-50) of eight wheat, four barley and four triticale cultivars were inoculated (Table 4.6) to give a diversity of potential host genetics. None of the wheat plants displayed any phenotype indicative of infection and, on testing, none were found to be hosting *Epichloë*. Infection phenotypes were observed in three of the barley cultivars ('Booma', 'Bumpa' and 'Dictator II/ D Hole') and two of the triticale cultivars ('Rufus' and 'Hawkeye') and examination by immunoblot (Methods 2.3.3) and fungus isolation (Methods 2.1.1) identified the phenotype-displaying grasses as hosts to *Epichloë*.

Table 4.6 Inoculation of wheat, barley and Triticale seedlings with strain AR3060

Cultivar/'line'	Cereal class	Number inoculated	Number infected	Floral (E+ plants)
'Wyalkatchem'	Wheat	50	0	N.A
EGA 'Gregory'	Wheat	50	0	N.A
'Empress'	Wheat	50	0	N.A

'Beaufort'	Wheat	48	0	N.A
'Axe'	Wheat	46	0	N.A
'Frame'	Wheat	43	0	N.A
'Gladius'	Wheat	38	0	N.A
'AGT Katana'	Wheat	38	0	N.A
'Booma'	Barley	35	1	Yes[1]
'Bumpa'	Barley	25	1	Yes[1]
'Dictator II/ D Hole'	Barley	21	1	No
'Emir'	Barley	38	0	N.A
'Rufus'	Triticale	47	12	Yes[2]
'Hawkeye'	Triticale	50	12	Yes[2]
'Winslow'	Triticale	5	0	N.A

[1] Subsequently determined to be tillers that had escaped infection
[2] no viable seed produced

The infection phenotype of barley cultivar 'Dictator II/D Hole' was stunted; and the plant remained vegetative, deteriorated and died prematurely. Barley cultivars 'Booma' and 'Bumpa' displayed infection as plants with small stature that were persistent and developed floral tillers. Subsequent examination of floral tillers showed that they had escaped infection while the vegetative parts of the plant remained infected. The triticale cultivars 'Rufus' and 'Hawkeye' infected with *Epichloë* displayed phenotypes similar to "Monad" wheat infected with strain AR3060. The infected plants were persistent and produced infected floral tillers but were developmentally retarded compared with un-infected plants (Fig. 4.6).

Figure 4.6 *Epichloë* **-infected and un-infected triticale. 'Rufus' triticale infected with** *Epichloë* **strain AR3060 (left) and un-infected (right).**

4.7 Inoculation of wheat and barley with loline producing strains from Eurasia

The infection result involving wheat and *Epichloë* strains AR3018 and AR3060 demonstrated that it was possible to infect a cultivated cereal grass with *Epichloë* and that different outcomes could occur when using different strains. One of the goals of this study was to achieve infection of modern cereal cultivars with endophytes that will produce metabolites that will give protection from insects and possibly abiotic stresses. The best phenotypic outcome with wheat came from infection with AR3060, but this strain is an ergovaline producer. Ergovaline production is an undesirable trait for cereal crops that

will be consumed by animals and humans. Lolines, peramine and chanoclavine however are not toxic to mammals and show activity against a number of invertebrate pest species (Pownall *et al.*, 1995; Fleetwood, 2007; Schardl, *et al.*, 2007; Berde & Schild, 2012; Finch, *et al.*, 2016). An experiment was performed,'strategy two', with the aim of infecting both wheat (cultivar 'Monad') and barley (cultivar 'Booma') with 10 loline producing strains (Table 4.7) sourced from *Elymus* (n=8) and *Hordeum* (n=2). Inoculations were performed with AR3060 at the same time, as a control.

Table 4.7 *Elymus* and *Hordeum* hosts of selected loline producing *Epichloë* strains. The basis of strain selection was the ability to produce loline alkaloids *in planta* and/or the position within SRR dendrogram clade and for maximum genetic dissimilarity within the range of strains available. NA=not analysed.

Strain	Source host	accession	peramine	chanoclavine	loline
AR3039	*Elymus caninus*	BZ 2679	√		
AR3046	*Elymus mutabilis*	BZ 4833	√		√
AR3064	*Elymus mutabilis*	BZ 4952	√		√
AR3067	*Elymus uralensis*	BZ 5083	√		√
AR3073	*Elymus caninus*	BZ 5510	√	√	
AR3078	*Elymus nevskii*	BZ 5592	√		√
AR3091	*Elymus sp.*	BZ 5813	√		√
AR3096	*Elymus fibrosus*	BZ 6937	√		√
AR3106	*Hordeum bogdanii*	BZ 8174	√		√
AR3108	*Hordeum turkestanicum*	BZ 6970	NA	NA	√

Fifty seedlings were inoculated with each of the 10 loline producing strains for both 'Monad' wheat and 'Booma' barley, and none of the seedlings became infected. The control inoculations with strain AR3060 resulted in eight infected 'Monad' wheat plants and one infected 'Booma' barley plant.

4.8 Inoculation of secondary gene pool *Hordeum* with *Epichloë* isolated from tertiary gene pool *Hordeum*

The genus *Hordeum* consists of three genepools (Von Bothmer *et al.*, 2003). The primary genepool consists of barley (*Hordeum vulgare*) breeding lines, cultivars and landraces and *H. spontaneum* (*H. vulgare* spp. *spontaneum*). The secondary genepool consists of *H. bulbosum* a species considered valuable for introduction genetic diversity into barley (Wendler *et al.*, 2015). *Epichloë* colonisation has not been described in primary and secondary genepool *Hordeum*. The tertiary genepool consists of ca. 30 species (Blattner, 2006) a number of which are known to host *Epichloë* (Wilson*, et al.*, 1991; Wilson, 2007; Card*, et al.*, 2014).

An experiment was peformed to test the ability of *H. spontaneum* to host *Epichloë* utilising five strains isolated from *H. bogdanii* and one strain isolated from *H. brevisubulatum* spp. *violaceum* (Table 4.8).

Table 4.8 Inoculation of secondary gene pool *Hordeum* (*H. spontaneum* spp. *spontaneum*) with *Epichloë* from tertiary gene pool *Hordeum* species

Hordeum vulgare spp. s*pontaneum*		
Epichloë source	inoculated	positive
Hordeum bogdanii	25	0
H. bogdanii	25	0
H. bogdanii	20	0
H. bogdanii	21	0
H. bogdanii	17	0
H. brevisubulatum spp. *violaceum*	25	15

Up to 25 *H. spontaneum* seedlings were inoculated with each of the tertiary genepool *Hordeum Epichloë* strains. Only one of the strains, the one isolated from *H. brevisubulatum* spp. *violaceum*, infected seedlings. Fifteen infected *H. spontaneum* plants were identified (Table 4.8). The infected plants displayed compromised phenotypes presenting no phenotypic advantage over *H. vulgare* infected germplasm.

4.9   Inoculation of wheat alien addition/substitution lines

The results obtained above suggested that successful inoculation of *Epichloë* endophytes into modern cereals could depend on the genetics of both the host and the endophyte strain. It may be that there are genetic factors in rye that enable infection/compatibility and/or factors in wheat and barley that prevent it. In an attempt to identify host genetic factors that impact infectivity and compatibility in wheat, wheat alien chromosome addition/substitution lines were inoculated with *Epichloë* and infection rates and phenotypes of infected plants were assessed.

An experiment involving the inoculation of over 1,500 seedlings was undertaken at the Arid Land Research Center (ARLC), Tottori, Japan, to examine infection frequency and infection phenotype of predominantly 'Chinese Spring' based wheat germplasm carrying alien chromosome introgressions/substitutions. AR3060 was the primary inoculant strain but AR3002 (isolated from *Elymus dahuricus*) and AR3067 (isolated from *Elymus uralensis*) were also included. Appendix 4 lists the 205 lines of 'Chinese Spring', 'Alcedo' and 'Vilmorin 27' wheats (*Triticum aestivum*) that were inoculated. These were carrying alien introductions from 18 species of *Aegilops*, *Agropyron*, *Elymus*, *Hordeum*, *Leymus*, *Psathyrostachys, Haynaldia* and *Secale*. Additional lines of *Triticum turgidum* and synthetic hexaploid wheats, representing a reconstitution of a hexaploid AABBDD wheat from the tetraploid (AABB) wheat 'Langdon' and *A. tauschii* (DD), were also inoculated. Based on immunoblot results, 95 of these lines became infected with *Epichloë*. One line, '512', a 'Chinese Spring' *Leymus* H substitution line was confirmed as infected and produced plants with normal phenotype and mature fully filled infected grain. Many of the other infected plants also had a normal phenotype and either produced uninfected grain (possibly due to escape) or showed seed transmission but with a delayed development which was exemplified by a 'stay green' phenotype and a protracted elaboration and maturation of floral spikes.

4.10   Infection of *Aegilops* with *Epichloë*

Aegilops is a Hordeeae genus closely related to wheat. The D genome component of hexaploid wheat (AABBDD) originates from *A. tauschii*. A screen of nine *Aegilops* species, including *A. tauschii*, failed to detect *Epichloë* (Chapter 3.4). An attempt was made to infect two species of *Aegilops*, *A. tauschii* (genome DD) and *A. biuncialis* (genome UUMM) (Hegde *et al.*, 2002). Twelve seedlings were inoculated with *Epichloë* strain AR3018. Seedlings were examined for infection by isolation (Methods 2.1.1). Mycelium grew from tissue isolated from two of the *A. biuncialis* inoculated seedlings (Fig. 4.7). Success in infecting *Aegilops* may offer possibilities in achieving infected synthetic wheat with desirable phenotypes.
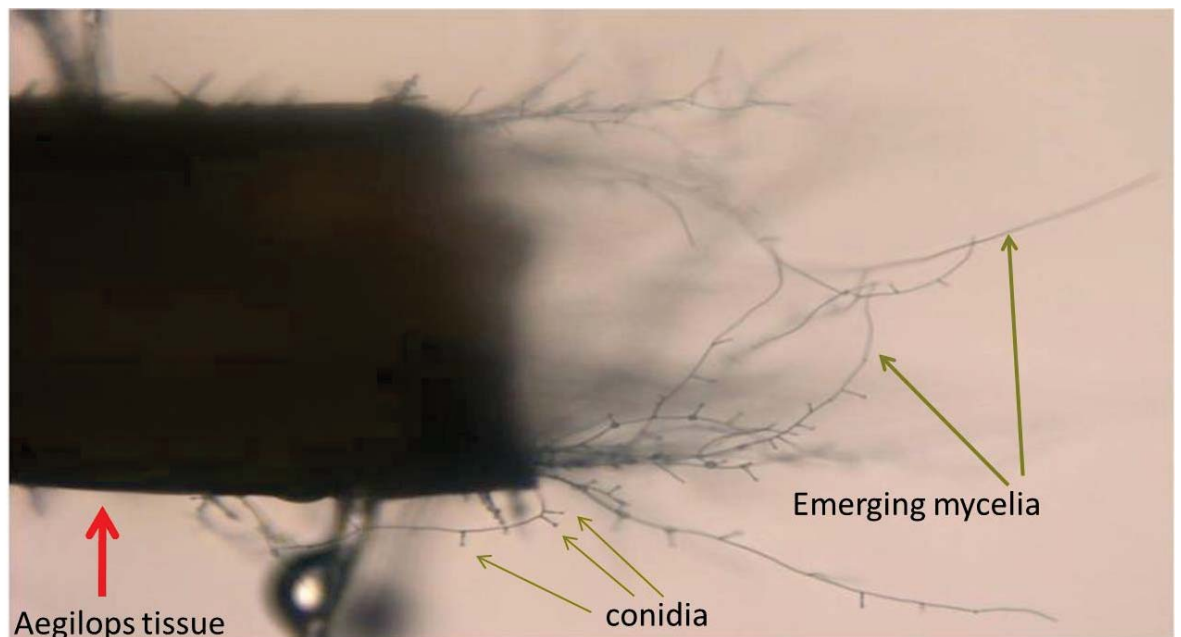
Figure 4.7 *Aegilops biuncialis* infected with AR3018. Imaged using a substage-illuminated dissecting microscope showing a silhouette of emerging hyphal strands and attendant conidia atop phialides

4.11 The formation of synthetic symbioses between Hordeeae grasses and *Epichloë*

There have been no reports of natural infection of modern cereal grasses such as wheat, barley and rye with *Epichloë* fungal endophytes. The experiments detailed here demonstrate that it is possible to achive synthetic infections using established inoculation techniques. The experiments outlined here suggest that the ability to achieve infection is dependent upon the *Epichloë* strain and the genotype of the potential host. Similary the genotypes of the symbionts determines the phenotype of the symbioses formed. The ability to manipulate these phenotypes by selecting for host genotype will be explored in the next section.

Chapter 5

5 Host and symbiosis phenotype

5.1 Introduction

The ability to isolate *Epichloë* from wild Hordeeae, such as *Elymus* and tertiary gene-pool *Hordeum*, and infect naïve cereal cultivar hosts, demonstrates a level of biological compatibility of the symbionts. However, beyond infection, the symbiosis involves ongoing intimate contact and co-ordinated growth between the two symbionts (Christensen *et al.*, 2008) and the maintenance of a combined homeostasis. Perturbation of this homeostasis has been reported when *Epichloë* have been genetically modified (Takemoto *et al.*, 2006; Tanaka *et al.*, 2006; Zhang *et al.*, 2006; Tanaka *et al.*, 2008; Charlton *et al.*, 2012; Johnson, *et al.*, 2013) but also, importantly, when grasses have been infected with *Epichloë* from other grass species (Christensen, 1995; Simpson & Mace, 2012c). The synthetic symbioses generated in this study have necessarily been generated by inoculation with *Epichloë* from species other than the destination host. Here, these synthetic symbioses demonstrated varying levels of incompatibility that manifested as changes in the morphological phenotypes of the infected host plants. In the case of rye, an obligate outcrossing species, the infection phenotype varied for individuals within a population. This was observed in plants both following primary infection and in the progeny of an infected plant.

5.2 Impact of *Epichloë* infection on the vegetative size of 'Rahu' rye plants

An experiment was performed to examine the range of vegetative plant heights of the progeny from pair crosses of *Epichloë*-infected plants with small, medium and large vegetative phenotypes. Un-infected plants were also identified with varying heights and these were crossed also. Populations of progeny infected with AR3002-type and AR3007

were sown and measured during their vegetative development (Fig. 5.1). It was hypothesised that small x small (sxs) crosses would give rise to small progeny and that large x large (LxL) crosses would produce large progeny. Of the three sxs crosses, two produced progeny that were all less than 30cm in height while the other sxs cross produced plants both less than 30cm and more (up to 40cm) than 30cm. Although crosses involving medium (m) and large plants (m x m, m x s and L x L) all, with one exception, produced plants more than 30cm in height (Fig. 5.1). With the exception of a single 'runt' outlier, the progeny of crosses between commercial E- (un-infected) plants all measured more than 30cm. It is important to note that the height of progeny of both E+ and E- crosses varied and that there was overlap between E+ and E- populations in the ranges of plant heights. This result would suggest that there may be scope for recurrent selection of large infected plants to move the phenotype to the large end of the range.

To test this, a further experiment was performed to examine the range of vegetative plant heights of a commercial un-infected population of 'Rahu' rye compared to a population, infected with an *Epichloë* strain - AR3002-type - that had been through several cycles of selection (Fig. 5.2). Populations were developed by selecting and crossing large phenotype plants from infected populations. These selections included plants from several families. The selected plants were then pollen-isolated together, or inter-crossed, and seed harvested from individual mothers. Plants were measured and recorded as family sets. The key observation in the aggregate data, indicated by red arrows in Figure 5.2, is that the height range, approximately 5-25cm, was similar for both infected and un-infected populations. Where the density of data points was highest there was more uniformity between the E- families than between the E+ families. There was a clear family effect on the range of plant heights and their frequency density (Fig. 5.2).
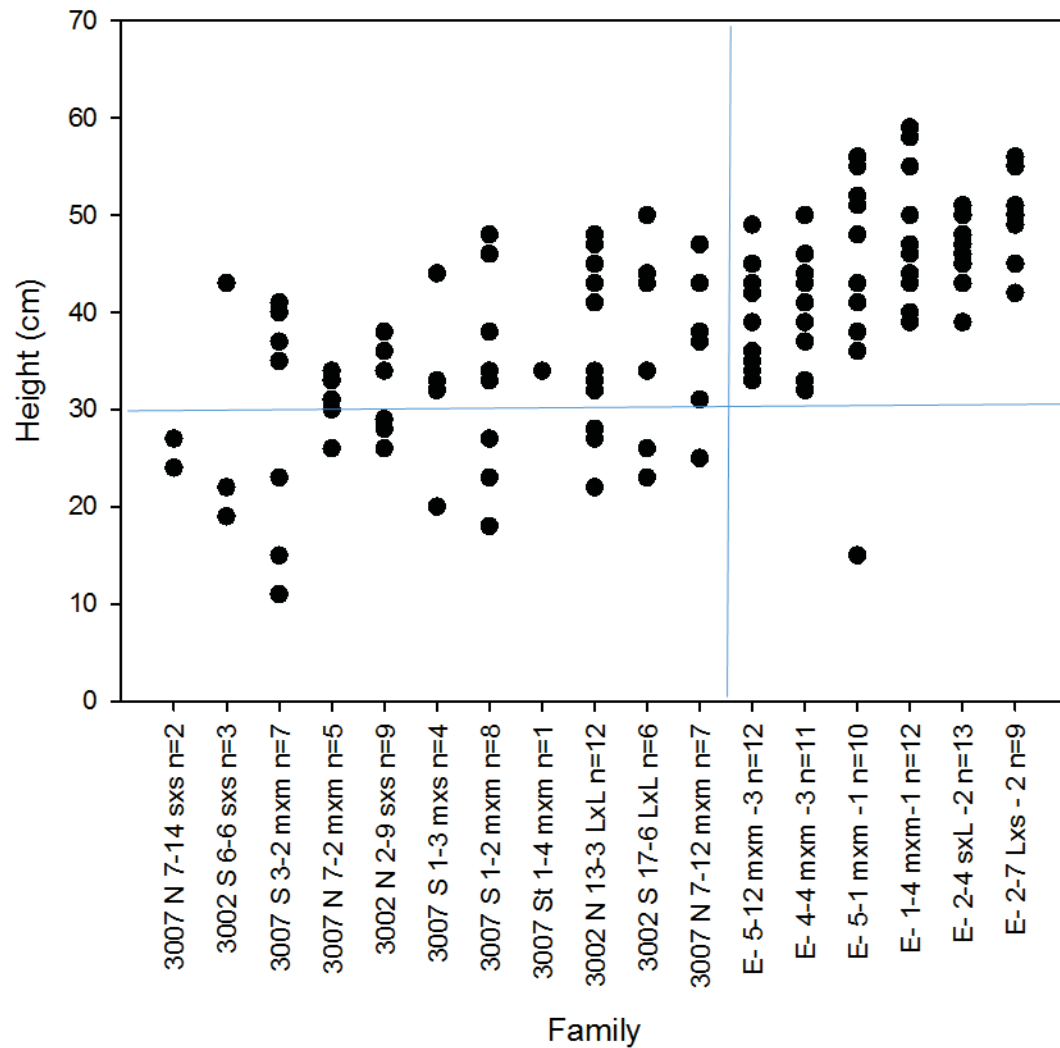
Figure 5.1 Pair crosses of plants infected with AR3002-type and AR3007 based on phenotype. Data points represent vegetative height (cm) of progeny plants grown from seed produced following crosses of plants either infected with the designated endophyte strain or endophyte-free (E-). Plants were crossed according to size – small x small (sxs), medium x medium (mxm), large x large (LxL), medium x small (mxs) and large x small (Lxs).
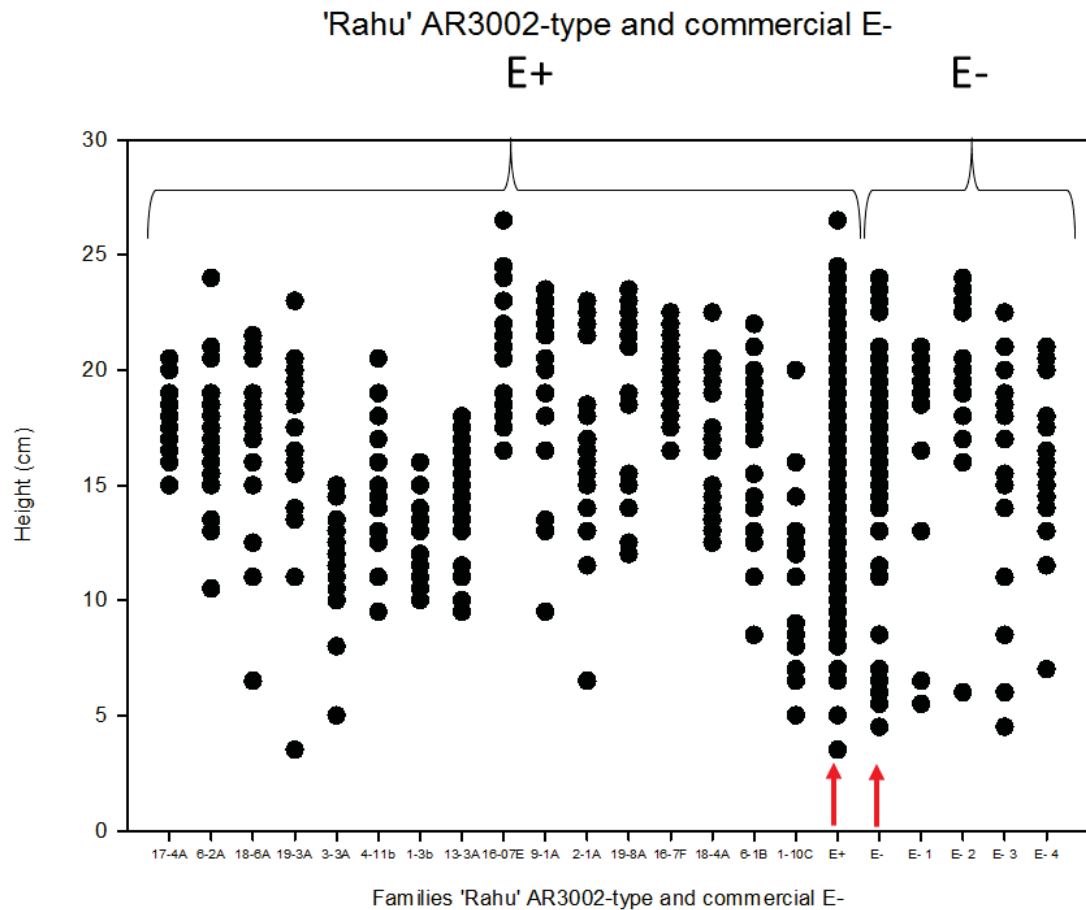
Figure 5.2 Plant heights of infected, AR3002-type, 'Rahu' rye following several cycles of selection for large plants and un-infected commercial 'Rahu'. The left-hand section of the x-axis shows the designation of the E+ mother plants (the numbers and letters denote family lineage) and the right-hand side the endophyte-free (E-) mother plants. Between these, indicated by red arrows, are the aggregate data of E+ and E- height measurements.

5.3 Infection phenotpyes in 'Rahu' rye showed family effects

When *Epichloë* strains were inoculated into rye (*Secale cereale*), an outcrossing species, individual genetically-distinct host genotypes showed morphological phenotypes that ranged from heavily stunted (sometimes terminal) through to some that resembled healthy uninfected plants. This phenotype spread was observed when 'naïve' germplasm was infected, and a similar range of phenotypes was also observed when progeny grain from infected plants were grown. The ranges and consistency of phenotypes showed apparent family-based patterns. An experiment was performed

whereby progeny grain from two different open-pollinated AR3068-infected 'Rahu' plants was sown. The progeny plants of one of the mothers showed a range of phenotypes, all smaller than un-infected individuals of the family set. The other mother produced progeny plants that were of a consistent phenotype, one that resembled the un-infected phenotype (Fig 5.3).
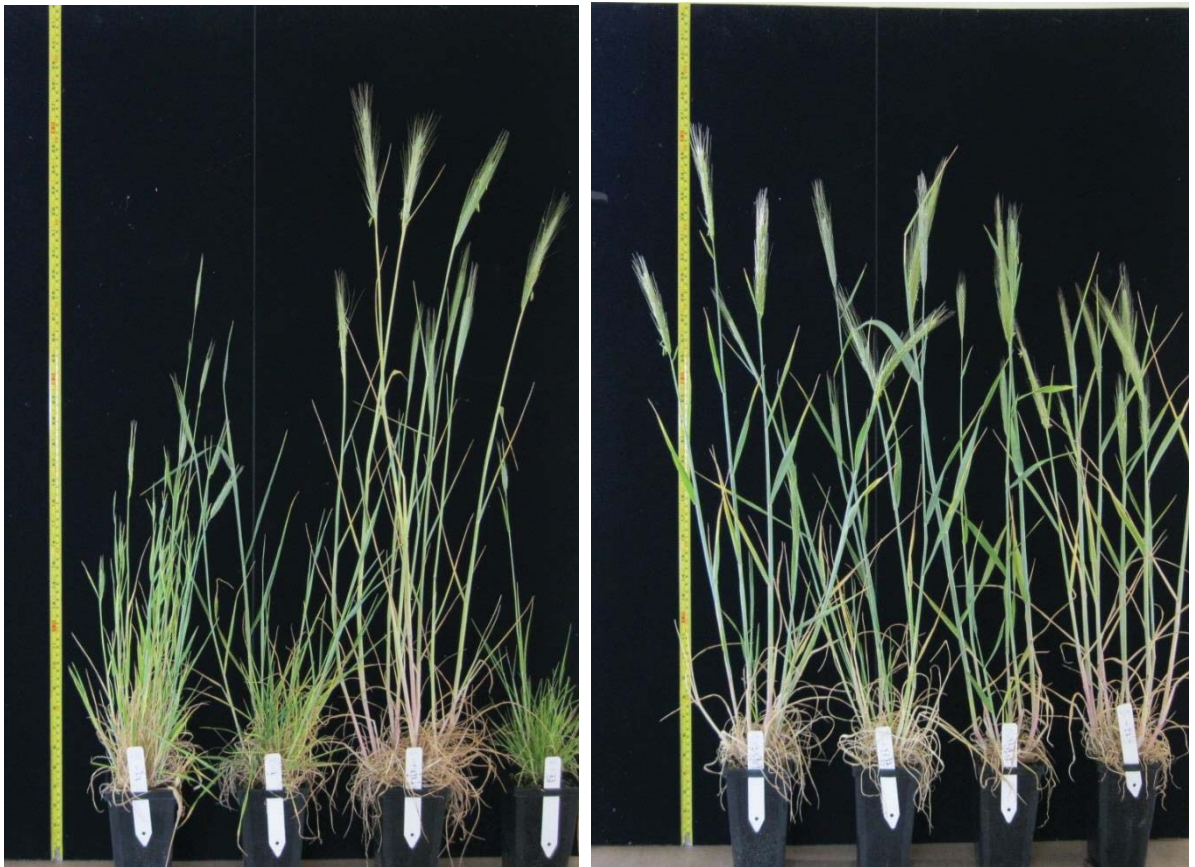
Figure 5.3 'Rahu' rye (*Secale cereale*) progeny from two individual open-pollinated infected mothers, i.e., two different families of 'Rahu' rye infected with an *Epichloë* strain sourced from *Elymus mutabilis* – strain AR3068. Left-hand image (family 1) left to right: infected, infected, un-infected, and infected. Right-hand image (family 2) left to right: infected, infected, un-infected, and un-infected. Plants shown were the same age and had been maintained under identical conditions.

5.4 *Epichloë*-infection affected rye seed but yields similar to un-infected rye could be achieved with recurrent selection

When a naïve population of rye was inoculated, the resulting infected plants varied in their morphological phenotypes. This effect of infection was observed in two rye cultivars, 'Rahu' and 'Amilo' (Results 4.2). In 'Amilo' rye, infection with *Epichloë* strain AR3018 resulted in a consistently poor and unsustainable phenotype whereas infection with strain AR3056 gave rise to a range of morphological phenotypes. This perturbation of the phenotype carried through to grain production, resulting in small, shrunken grain (Fig. 5.4)

Figure 5.4 Grain of *Epichloë*-infected (AR3056 - E+) and un-infected (E-) rye (*Secale cereale*). Left: 'Rahu' E-, middle: 'Amilo' E- and right: 'Amilo' infected with *Epichloë* strain AR3056. Note the well filled grain of E- samples with blue/greenish tinge compared to shrivelled light brown grain of the E+ sample

Similarly, rye infected with a range of other strains; AR3002, AR3005, AR3007, AR3017, AR3042, AR3071 and AR3074, displayed not only vegetative phenotype effects but also reproductive effects manifested in grain fill. Recurrent selection of these infected rye using infection status and morphological phenotype as selection criteria led to an improvement in both plant stature and grain fill and resulted in infected progeny with seed yields comparable to un-infected plants (Fig. 5.5). Both infected and un-

infected plants showed considerable variation in total seed yield. The yield data here were taken from *Epichloë*-infected populations that had different numbers of selection cycles. Progeny from plants with four rounds of selection, AR3002, AR3005 and AR3007 had higher rates of grain infection than those from populations with three rounds of selection, AR3017, AR3042, AR3071 and AR3074. Seed yields from individual mothers differed both for infected and un-infected plants but there was no clear bias for yield level correlated with infection status (Fig. 5.5).
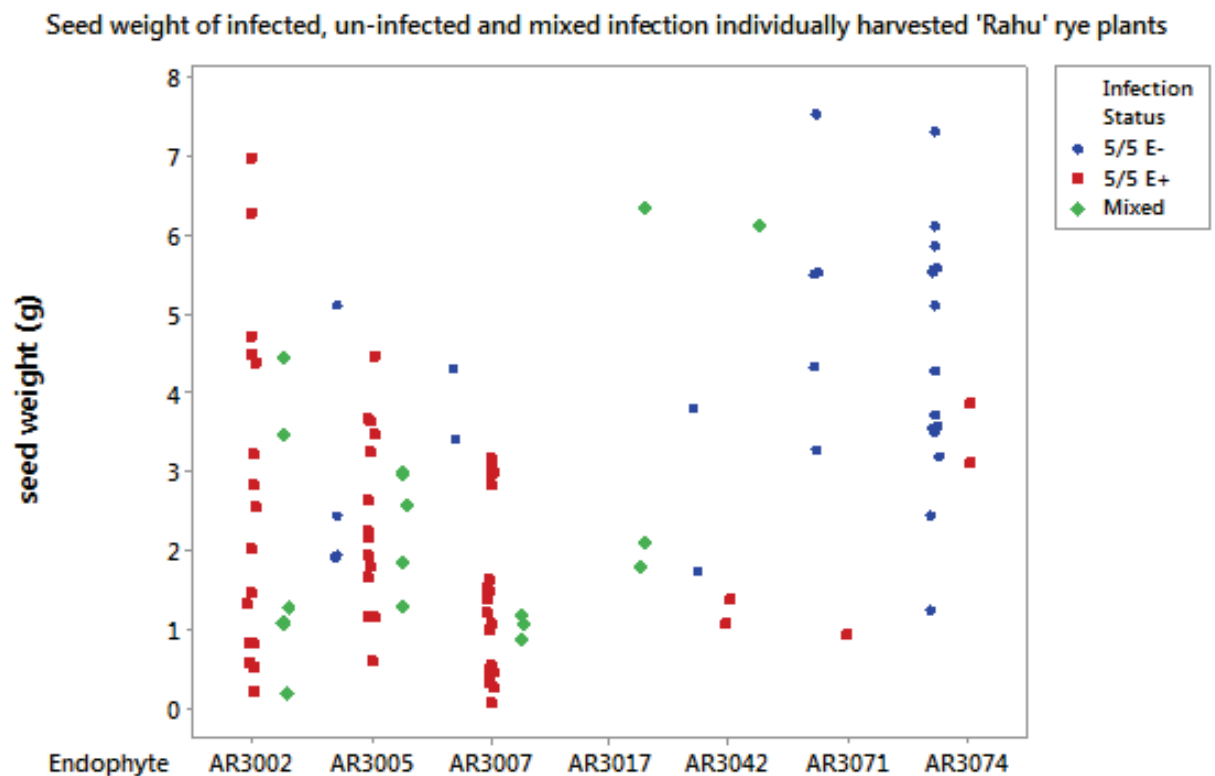
Figure 5.5 Seed weight of infected (E+), un-infected (E-) and mixed infection individually harvested 'Rahu' plants. The yields of individual plants ranged from 0.09g – 7.53g. The yields from infected and un-infected plants across strain sets covered similar ranges.

5.5 Field grown 'Rahu' AR3002-type produced more heads on plants of similar average height to un-infected 'Rahu'

In the spring of 2013, a set of progeny grain of 'Rahu' infected with an AR3002-type strain was sown to generate plants for isolating as a population. Plants were immuno-blotted to determine infection status. As plants developed, large endophyte-infected plants were selected for two isolations. 'Elite 1 early' was a line made up of plants from a population with a high endophyte transmission rate, and 'Elite 2 early' was made up of material from a population with relatively lower endophyte transmission. An un-infected (nil) line was sown as a control isolation. Plants, including the nil control, were arranged in groups under pollen exclusion tents to cross pollinate within their sets. At harvest, in the summer of 2014, the number of heads on each plant was counted and the height of each plant was measured (Table 5.1). The average number of heads was higher in the two infected populations, with means of 13.6 and 13.1 per plant, than the nil population, with a mean of 9.5 per plant. The ranges were also higher in the infected populations n=26 and 25, compared to the nil n=20.

The mean height difference of infected compared to un-infected populations was 0.1-0.2m, with mean heights of infected populations being 1.4 and 1.3m, compared to the nil population which was 1.5m. However, the height range of the infected populations (0.81 and 0.82m) was twice that of the nil population (0.41m).

Table 5.1 Poly-cross pollen isolations of 'Rahu' AR3002-type and nil

Line	No. Heads average	Height (m) average	No. Heads range	Height (m)
Elite 1 early	13.6	1.4	6-32 (26)	0.93 - 1.74 (0.81)
Elite 2 early	13.1	1.3	3-28 (25)	0.83 - 1.65 (0.82)
Rahu nil	9.5	1.5	4-24 (20)	1.23 - 1.64 (0.41)

5.6   Harvest Index

The harvest index (HI), within the context of cereal crops, is a measure of the ratio of grain yield to biological yield or biomass. The values for modern varieties of most intensively cultivated grain crops fall within the range 0.4-0.6. HI is considered to have high heritability with a weak response to variation in environmental factors (Hay, 1995).

An experiment was performed on a subset of field grown spaced plants at Lincoln New Zealand, commencing in the autumn of 2015 and harvested in the summer of 2016 (Fig 5.6 centre panels).

Figure 5.6 'Rahu' AR3002-type and E-. Top left: plants at harvest 2012, pot grown isolated under pollen exclusion tents. Bottom left: plants at harvest 2015, pot grown isolated under pollen exclusion tents. Middle: top, AR3002-type-infected and bottom, nil field grown plants at Lincoln 2016. Right: close-up of AR3002-type infected 'Rahu' rye field grown at Lincoln 2016.

Plants were planted on a 50cm grid and overhead irrigation was applied as required, dependent on natural rainfall. Nitrogen was applied twice, once in October and once in November at the rate of 90kg/ha and a combination broadleaf herbicide/fungicide

(Trimec 3L/ha and Quantum 200ml/ha) was applied in October. The trial consisted of 'Rahu' rye infected with each of eight *Epichloë* strains (a total of 780 plants, Table 5.2) and a control set of 146 un-infected plants. Plants were grown side-by-side in a single isolation block.

Table 5.2 Endophyte-infected 'Rahu' spaced plant trail. Numbers of plants of each endophyte strain.

AR3002	AR3005	AR3007	AR3042	AR3068	AR3071	AR3074	AR3002-type	Total
134	118	147	36	95	1	35	214	**780**

Immediately prior to harvest five large and five small individuals were selected, by eye, from each strain set, (except AR3071), and measured for total height and spike number. These plants were then harvested by severing at ground level. Seed was threshed from each individual, seed and chaff was oven dried and weighed and an HI calculated (Table 5.3).

Table 5.3 Harvest Index of spaced plant 'Rahu' rye (*Secale cereale*) un-infected and infected with each of eight *Epichloë* strains. n=10 for all except AR3071 n=1

	Top value	mean	median
Nil	0.45	0.36	0.36
AR3005	0.45	0.30	0.32
AR3002	0.39	0.25	0.29
AR3068	0.38	0.27	0.26
AR3074	0.38	0.16	0.15
AR3042	0.33	0.26	0.27
AR3002-type	0.30	0.24	0.25
AR3071	0.23	0.23	0.23
AR3007	0.23	0.14	0.13

The top HI values from each strain set ranged from 0.23 (AR3007) – 0.45 (AR3005 and Nil). The average values of the *Epichloë* -infected plants are tempered by the fact that both large and small plants were included in the measure. Heights of AR3005 plants sampled, for example, ranged from 0.6-1.3M (0.7M range), while heights of Nil plants ranged from 1.4-1.5M (0.1M range). The mean (and median) HI values of five plants

from each end of the size spectrum of each strain population were calculated. The mean HI value of the large plants was higher than the small plants for all of the infected 'Rahu' except for those infected with AR3007 (Table 5.4). In the un-infected (nil) population, small plants had a higher mean HI than the large un-infected plants.

Table 5.4 Harvest index of large and small plants from *Epichloe* -infected populations of 'Rahu' rye. Values are the mean HI of five large and five small individuals from each strain population. Median HI value in brackets.

	Large plants n=5	Small plants n=5
Nil	0.35 (0.34)	0.38 (0.36)
AR3005	0.37 (0.38)	0.23 (0.23)
AR3002	0.32 (0.31)	0.18 (0.24)
AR3068	0.30 (0.29)	0.23 (0.24)
AR3074	0.21 (0.15)	0.12 (0.11)
AR3042	0.27 (0.29)	0.26 (0.27)
AR3002-type	0.27 (0.28)	0.20 (0.22)
AR3007	0.12 (0.12)	0.16 (0.15)

5.7   *Epichloë*-infection phenotypes in self-fertile rye

Having observed the range of infection phenotypes displayed in 'Rahu', a genetically diverse outcrossing rye population, it was hypothesised that infecting a more genetically narrow population would result in less diversity of the infection phenotype. An experiment was undertaken to infect a selfing rye line, Lo6-N and its cytoplasmic male sterile (cms) analogue, Lo6-P (Chapter 4.4). The infected populations of both lines were more diverse than the un-infected populations with the largest phenotypes equivalent in stature to un-infected plants (Fig. 5.7).
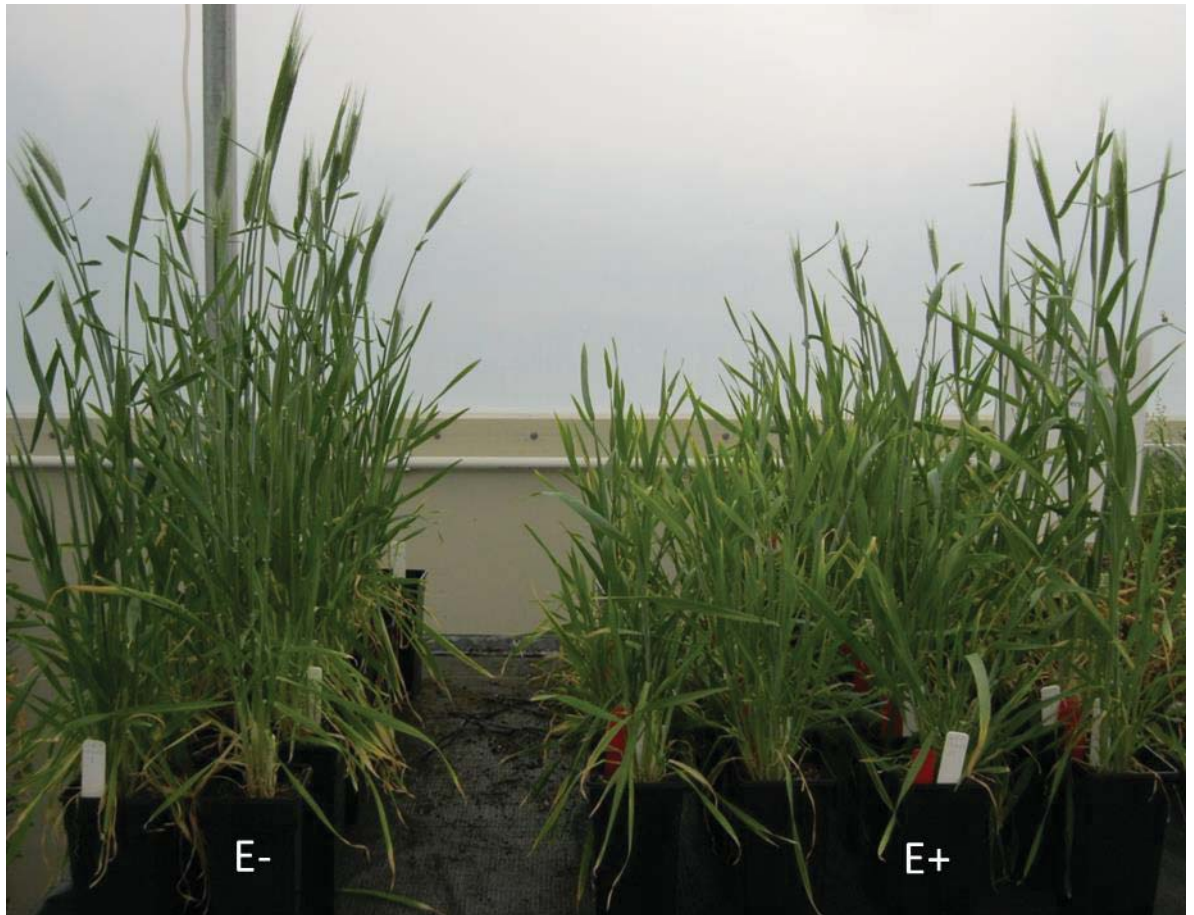
Figure 5.7 KWS selfing rye un-infected (left) and infected with AR3002-type *Epichloë* (right). Note the uniform development of the un-infected plants consistent with a selfing line. Infected plants varied in their stage of development with some (far right) at a similar stage to the un-infected plants.

With the exception of two outliers in the Lo6-P E- population, the variance of the E- lines was much lower than the E+ (Fig. 5.8). A portion of the infected populations overlapped in stature with the range of the E- populations (boxed area Fig. 5.8). This large range of infection phenotypes (plant height) compared to un-infected plants was consistent with observations in the outcrossing rye 'Rahu' (Chapter 5.2). Again, there were broad regions of overlap between the infected and un-infected populations in the phenotypes of individuals and the range of those individual phenotypes.
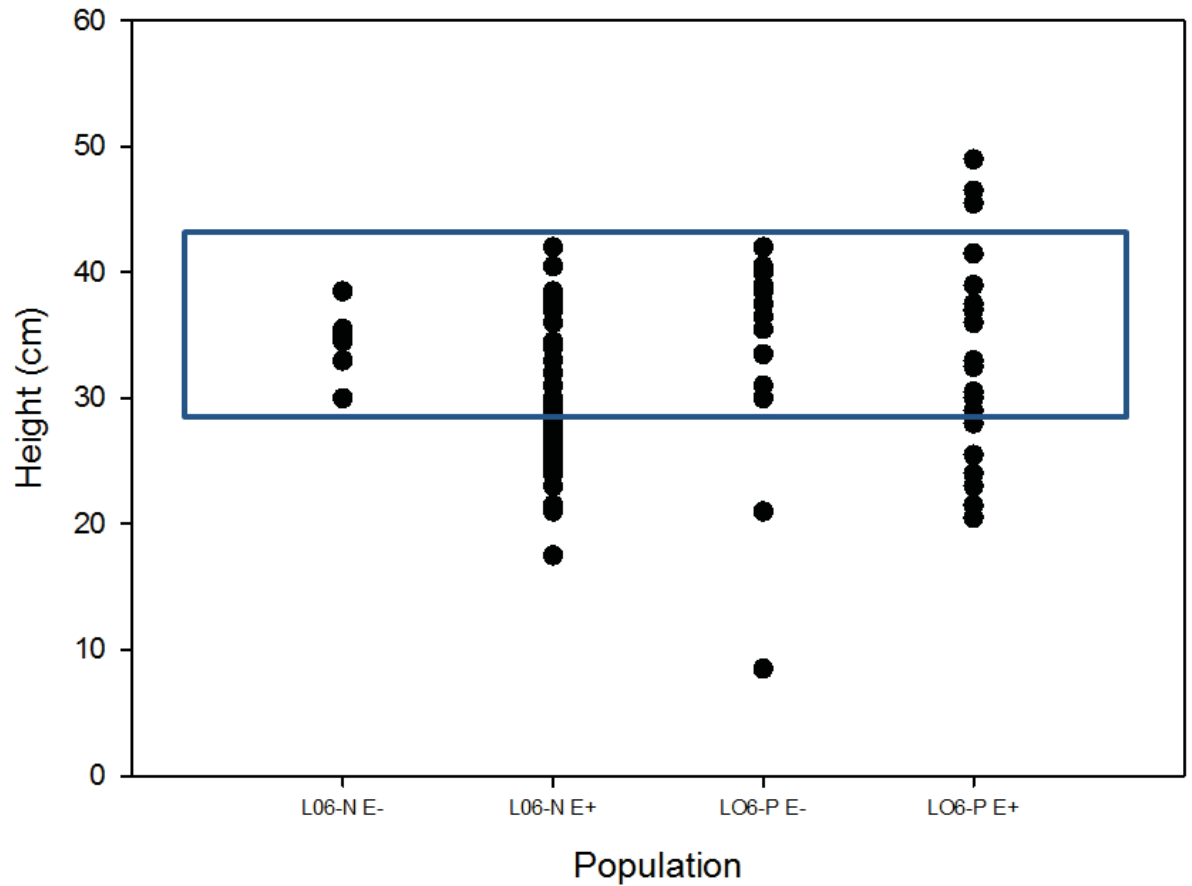
Figure 5.8 Stature of AR3002-type *Epichloë*-infected and un-infected selfing rye lines Lo6-N and Lo6-P. Infected lines showed a much broader height range compared to the un-infected lines. The boxed region illustrates the height range common across line and infection status.

Although all of the selfing rye plants developed to a stage where they had floral tillers, the development of infected plants was delayed compared to the un-infected plants. Infected and un-infected plants produced similar numbers of floral tillers, but the un-infected plants reached BBCH stage 59 before infected plants (Fig. 5.9).

KWS rye number of floral tillers
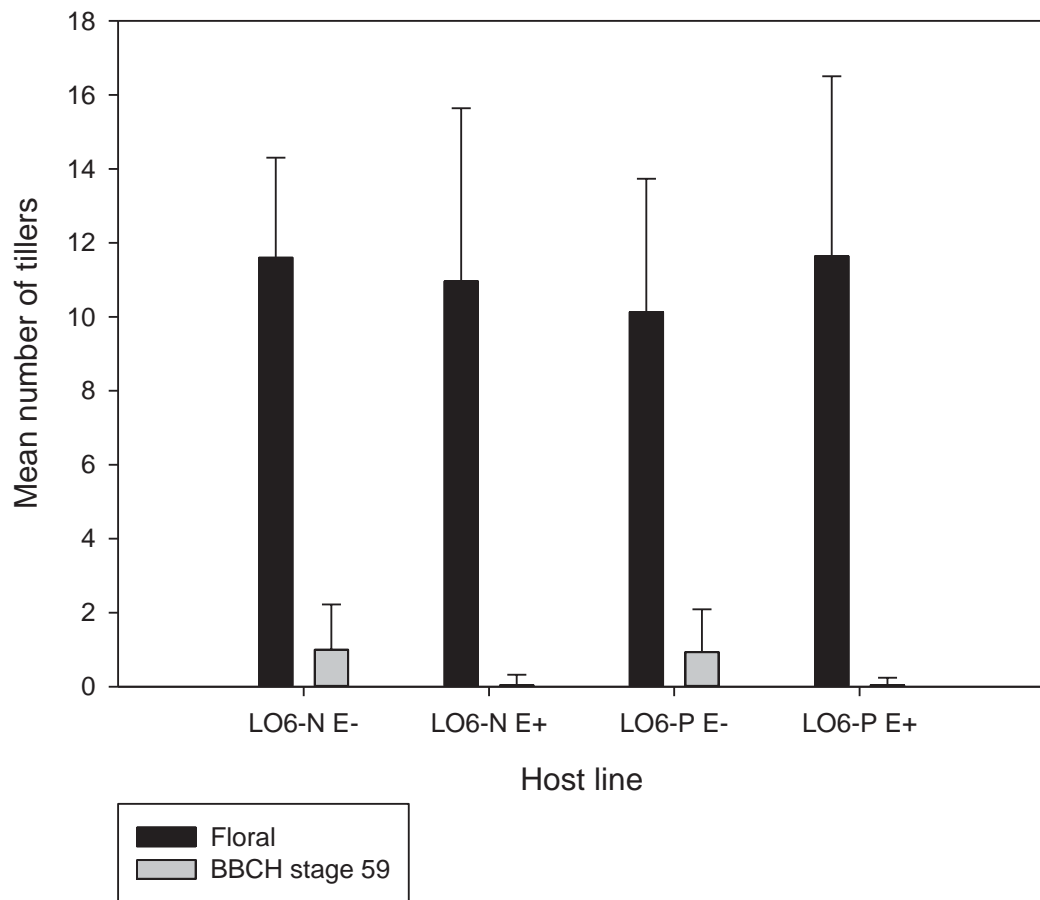
Figure 5.9 Floral tiller number and development stage on infected and un-infected selfing rye (KWS rye). In addition to the enumeration of floral tillers, a phenological development assessment was made according to the BBCH-scale for cereals. BBCH stage 59 describes the end of heading when the inflorescence is fully emerged. Bars show standard error of the mean.

Figure 5.10 Examples of KWS selfing rye infection phenotypes. Plants infected with *Epichloë* **strain AR3002-type. From left to right: late, middle and early flowering individuals. Although individuals differed in stature and stage of development at the point in time when records were made, all plants eventually developed floral tillers**

5.8 Crossing of *Epichloë*-infected selfing rye

Infected plants were separated into three sets based on early, mid or late heading (Fig. 5.10). Plants at a similar heading stage were isolated under bags constructed from pollen-proof fabric (Fig. 5.12). Male sterile Lo6-P plants were isolated one on one with male fertile Lo6-N plants, also multiple Lo6-P plants were isolated with a single Lo6-N pollinator in addition to isolations of multiple male fertile Lo6-N plants as outlined in Figure 5.11.
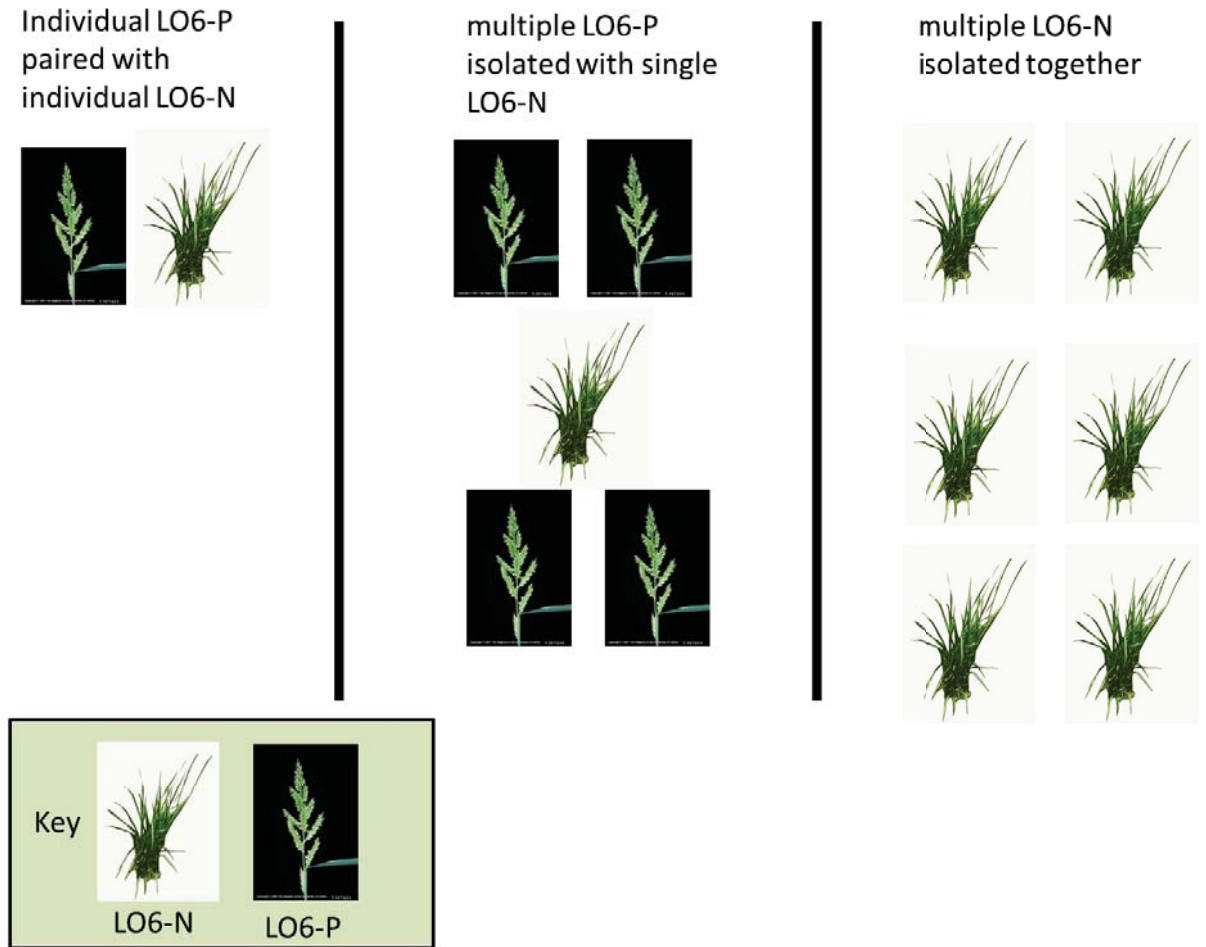
Figure 5.11 Schematic of isolation of KWS selfing rye. Three isolation strategies were adopted: (a) Pair-crosses between a cms, LO6-P, and a male fertile, LO6-N (maintainer) plant, (b) crosses with multiple cms plants and a single maintainer and (c) a population of maintainer plants

Figure 5.12 Isolation of selfing rye, infected with *Epichloë* AR3002-type, showing arrangement of plants. Infected plants at a similar stage of development were grouped together. Floral heads were covered with bags constructed from pollen-proof fabric and tied at the base.

5.9   Field grown *Epichloë*-infected 'Rahu' displayed a larger height range than un-infected 'Rahu' at harvest

In the spring of 2014, seed harvested from 'Rahu' infected with *Epichloë* strains AR3068 and AR3074 were sown for later planting in the field at Lincoln, Christchurch. The seedling progeny were examined for the presence of endophyte using an immuno-blot assay (Methods 2.3.3). Plants were planted on a 50cm grid and overhead irrigation was applied as required, dependent on natural rainfall. Nitrogen was applied twice, once in October and once in November at the rate of 90kg/ha and a combination broadleaf herbicide/fungicide (Trimec 3L/ha and Quantum 200ml/ha) was applied in October. The primary aim of the planting was to increase seed of infected plants. A stature difference was evident between plants in the E+ and E- plots, with more variation evident in the E+ plants. This observation was consistent with infection phenotypes observed in glasshouse studies both with outcrossing 'Rahu' and the selfing rye from KWS Germany. Plants were measured in December 2014 prior to their harvest in early February 2015. Although the infected population showed high variance with a height range of over 100cm compared to a 20cm range in the E- population, a portion of the E+ plants were of similar height to E- plants (Fig. 5.13).

'Rahu' AR3068 and AR3074 at Simpson's block Lincoln
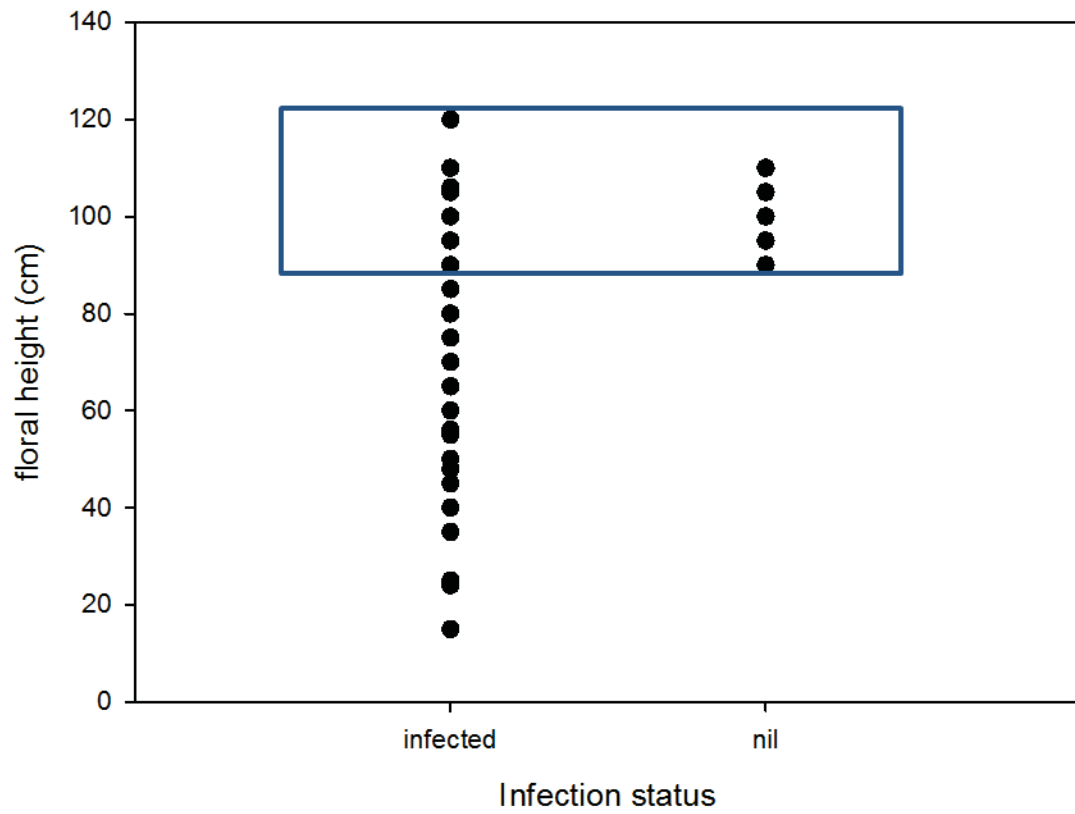
Figure 5.13 Plant height of field-grown 'Rahu' infected with strains AR3068 and AR3074 and endophyte-free (nil) immediately prior to harvest. As with vegetative heights in glasshouse-grown plants, *Epichloë*-infected plants displayed a larger range of heights compared to un-infected plants

5.10 Field-grown *Epichloë*-infected 'Rahu' showed different rust mean leison scores

Immediately prior to harvest in the summer of 2015, plants in the field at Lincoln were

visually scored for the presence of leaf rust (*Puccinia* spp.) using a 0-5 scale. On the

scale, 0 was assigned where there was no rust present and 5 where there was a heavy

infestation. None of the plants scored had no rust. The number of plants examined

differed between E+ (n=40) and E- (n=11). The score distributions differed between the

two populations. No '5' was scored in the E+ population but one was scored in the E-

population. No '1' was scored in the E- but three were scored in the E+. The magnitude

relationship of scores 2 and 3 were inverted between the E+ and E- populations. The E+ population had more 2s than 3s whereas the E- had more 3s than 2s (Fig. 5.14). A repeat scoring the following season, 2016, was not possible due to low disease incidence.
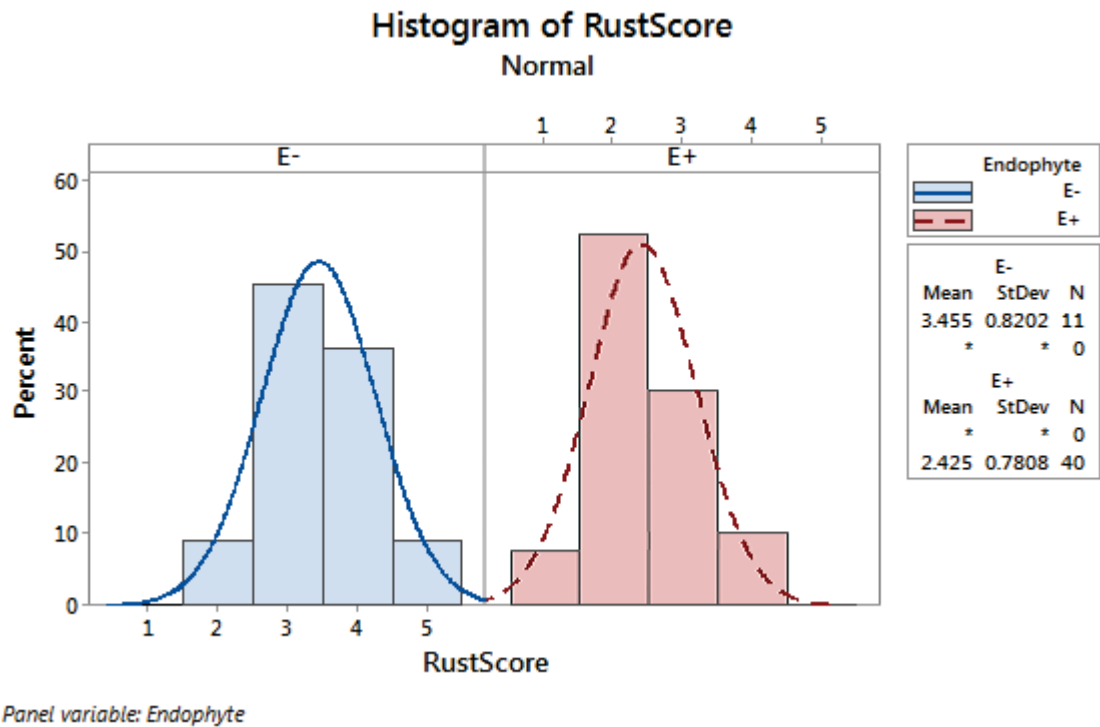
Figure 5.14 Leaf rust (*Puccinia* spp.) scores – 'Rahu' E+ (AR3068, AR3074) and E- (endophyte-free). The E+ population did not score a 5 (high infestation) and scored more 2s than 3s. The E+ population scored one 5 and more 3s than 2s. Unpaired *t* test P value <0.001.

5.11 Infected rye seed maintained endophyte viability when stored

An experiment was performed to examine *Epichloë* viability in stored grain. A bulk was made using 20 grain from each of 26 AR3002-type 'Rahu' accessions held in the MFGC. For the period of the experiment the bulked seed was stored indoors at ambient temperatures in an office room heated during the day. Forty (40) grain from this bulk were sown every month from May through to Novemember and isolations were made from 5-7 day old seedlings to determine viable infection. Not all seed germinated, however *Epichloë* was isolated from all germinated seedlings (Fig. 5.15).

Endophyte viability following ambient seed storage
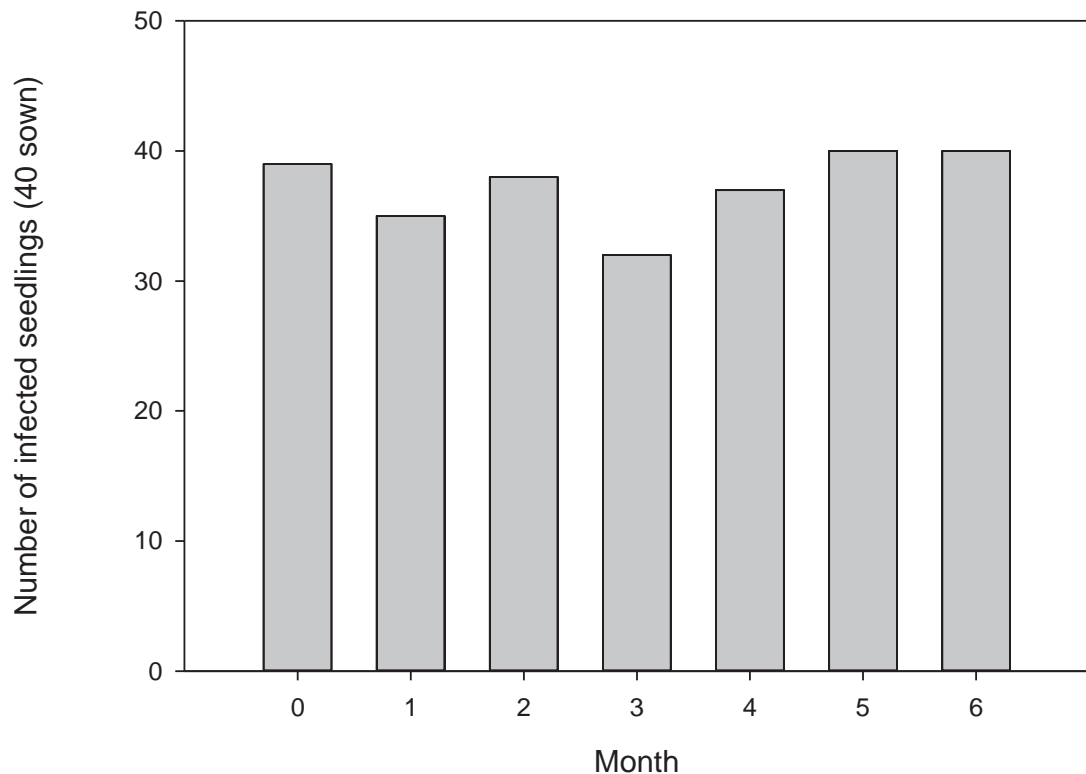
Figure 5.15 Viability of *Epichloë* in stored seed. Number of infected seedlings generated from n=40 seed sown every month following storage at room temperature from May (month 0) – November (month 6).

5.12 Synthetic Hordeeae grass/ *Epichloë* symbiosis phenotype

Although it is possible to produce synthetic Hordeeae grass/ *Epichloe* symbioses via inoculation with cultured fungus, the symbiosis phenotype outcomes vary. The experiments conducted here demonstrate that variation in host plant genetics can be exploited to drive symbiosis phenotype toward that resembling un-infected germplasm with regard to vegetative morphology and seed yield. Addtionally, *Epichloe* transmission both vegetative, and to the seed, can be improved through selection of infected host germplasm

Chapter 6

6 Discussion

This research was focused on a host specific symbiont endophytic fungus, *Epichloë*, and its ability to form synthetic symbioses with cultivated cereals. A screen of wild grass populations was performed to gather together a collection of *Epichloë* strains to act as inoculum for the establishment of synthetic symbioses with cultivated cereal grasses. These strains were genetically and chemically characterised to assess their suitability as novel symbionts. A feature of the symbioses synthesised in this study was the effect of infection on the host morphological phenotype. Experimentation performed in response to this observation demonstrated the ability to select desirable host genotypes from within infected populations.

6.1 *Epichloë*: filamentous fungal endophytes that form symbioses specifically and exclusively with grasses of the Pooideae sub-family

Epichloë endophytes are grass-associated fungi with close relatives that are insect pathogens. There is good evidence that the common ancestor of the grass symbionts within the fungal family Clavicipitaceae, that includes *Epichloë*, originated via a number of inter-kingdom host jumps from a fungus that colonised insects. The Clavicipitaceae includes colonisers of animals, plants and other fungi (Spatafora *et al.*, 2007). An examination of the cladogeneisis of the grass sub-family Pooideae shows concurrence with that of *Epichloë,* suggesting concomitant origins of the symbionts (Schardl *et al.*, 2008). The Pooid grasses are estimated to have differentiated around 40 million years ago (Glémin & Bataillon, 2009). It may be that an early Pooid grass became infected with an insect-associated fungus and the two organisms co-evolved giving rise to the array of *Epichloë* and Pooid grass species that we see today as Schardl et al (2008) have suggested,

a Pooid progenitor may have become infected with an insect-associated fungus and the capacity of *Epichloë* to confer drought tolerance and herbivore (both mammalian and invertebrate) resistance facilitated a habitat shift of the grass from shady to open areas, contributing to the further differentiation of Pooideae. Today *Epichloë* are associated solely with Pooideae grasses. The Pooideae consist of around 200 genera covering over 4,200 species circumscribed by 14 tribes. *Epichloë* have been observed in many, but not all, of the grasses that have been examined within the sub-family,. Despite this long period of co-evolution, the observation of *Epichloë* in their hosts and the appreciation of their significance both biologically and agriculturally has been comparatively recent. Reports in the literature concerned with the biology of *Epichloë* span less than 150 years while those concerning animal toxicity in pasture grasses no more than 50 years.

Research in the agricultural sphere has been driven by the need to address animal toxicity issues. This research has been conducted primarily in the USA with the ergovaline producing *Epichloë coenophialia*/tall fescue association and in NZ with the ergovaline and Lolitrem B producing *Epichloë festucae* var *lolii*/perennial ryegrass association. The solution to the issue of *Epichloë*-produced toxins, such as ergovaline and lolitremB, in pastures grasses has been enabled by two features of *Epichloë* biology. First, the fact that strains within a species can differ in their alkaloid metabolite profile and second, that it is possible to inoculate naïve germplasm with cultured fungus and establish a synthetic symbiosis. The diversity of alkaloid profiles has allowed the screening of natural populations of *Epichloë* for the presence of strains that do not produce mammalian toxins, while still producing metabolites that confer insect pest protection to host grasses. These strains could then be isolated and inoculated into elite pasture cultivars, establishing symbioses that were subsequently seed-transmitted and could be delivered to agriculture through proprietary pasture seed. The thesis of this study is that the approaches and

practices deployed in pasture grass systems might be able to be applied to cereal grasses and that similar agricultural benefits might be accrued to cereal production systems. Understanding the evolution, biology and host associations in natural systems would aid the pursuit of this approach. Recurrent selection of germplasm is effectively evolution sped-up and so offers a method of improving symbiosis over relatively short periods.

6.2 Screening of natural populations of Hordeeae (Triticeae) grasses *Elymus* and *Hordeum* showed presence of *Epichloë*

Given that modern cereal grasses such as wheat, barley and rye did not host *Epichloë*, it was necessary to source strains from other Pooid grasses for use in attempts to achieve synthetic infection. Prior screening of Pooid grasses of tribe Hordeeae, sourced from a number of international germplasm centres, for the presence of *Epichloë*, identified infections in *Elymus* and tertiary gene-pool *Hordeum* species and so these grasses formed the basis of a screen to identify *Epichloë* (Results Chapter 3). Many of the infected *Elymus* identified from this screening were originally sourced from China and bordering regions. In light of this, a targeted collection trip was made in Gansu Province, North West China. One of the key observations in this part of the study was the difference in viable *Epichloë* infection frequencies between *ex situ* (germplasm-centre sourced) and *in situ* (field sourced) collections. Approximately half (49%) of the *in situ* accessions were viably infected, while only 13 % of the *ex situ* accessions were infected. The viability of *Epichloë* in seed is known to decline over time, especially under warm and humid conditions (Hume *et al.*, 2013). It is likely that this difference in viable infection frequency reflects the fact that the *ex situ* material had been stored for periods of years while the *in situ* material was germinated and screened soon after collection. Another key observation was the extent of the genetic diversity of the *Epichloë* collected in Gansu Province. Using 25 marker SSR analysis 20 genetically distinguishable strains were identified in a sample of

99 *Epichloë*-infected *Elymus* (section 3.3). This diversity of *Epichloë* likely reflects the diversity of the *Elymus* host in China. *Elymus*, especially the StY genome species, known also as *Roegneria*, have a centre of diversity in China (Baum, *et al.*, 1991). The co-evolutiuonary hypothesis, of *Epichloë* and its host grasses (Schardl, *et al.*, 2008), would suggest that where there is a diversity of host genetics, there might also be a diversity of symbiont genetics.

6.3 *Epichloë* sourced from *Elymus* and tertiary gene-pool *Hordeum* produced similar alkaloids to *Epichloë* from pasture grasses, but not Lolitrem B

Several alkaloidal secondary metabolites produced by *Epichloë* have been well characterised, especially the ergot alkaloids ergovaline and chanoclavine, the indole diterpenes including Lolitrem B, the lolines and the non-ribosomal peptide synthetase (NRPS) metabolite peramine. A High Performance Liquid Chromotography (HPLC) and Mass Spectrometry (MS) analysis of *Epichloë*-infected *Elymus* collected from Gansu Province, China, showed the presence of all four classes. No lolitrem B was detected, however the indole diterpenes (IDTs) paspaline and terpendole I were detected, with the presence of peramine being common across the accessions collected (section 3.2). Early pathway IDTs such as paspaline and terpendole do not present the animal toxicity issue that Lolitrem B does. The presence of alkaloids similar to those identified in pasture grass populations allows a similar selection strategy for strains to be used in cereal grasses, namely the selection of strains producing insect deterrent and/or toxic alkaloids such as peramine and lolines that do not produce mammalian toxins such as ergovaline or lolitrem B. Strain selection for novel cereal associations is further tempered by differential strain phenotype effects, this not a primary issue in pasture-grass systems.

6.4 Synthetic infections of wheat, barley and rye could be achieved with *Epichloë* sourced from wild *Elymus* and *Hordeum*

A primary aim of this research was to attempt to synthetically infect cereal grasses with *Epichloë* with a view to realising the benefits of infection that have been well documented in pasture grasses. It has been demonstrated that rye, wheat and barley could all be systemically infected with *Epichloë* (Results Chapter 4) but there were impacts of infection on the morphological phenotype of the novel hosts. A key observation was that the nature of the infection-induced phenotypes differed between the outcrossing species rye and the inbreeding species wheat and barley. While the *Epichloë*-infected wild *Elymus* and *Hordeum* grasses showed no apparent symptoms of infection, the cultivated cereal grasses inoculated with the *Epichloë* isolated from these wild grasses showed outward signs of infection (Results Chapter 5).

Not all grasses inoculated became infected. The ability to form a synthetic symbiosis was influenced by the genotype of the fungus and the genotype of the inoculated plant.

6.5 Infection of modern cereals with *Epichloë* sourced from other species resulted in altered morphological phenotypes

The production of *Epichloë*-infected pasture grass populations that are not toxic to grazing animals, but retain insect pest resistance, has typically involved isolating *Epichloë* from the same grass species as the targeted novel host. As such, the performance of the endophyte with regard to colonisation of daughter tillers via axillary buds and transmission to progeny via seed, has differed little from that seen in the original host. Additionally, the asymptomatic nature of anamorph-typified *Epichloë* infection has been retained in the novel host. The situation has occurred however, where an *Epichloë* was isolated from one grass species and used to synthetically infect a a different species. For example, loline-producing fescue endophytes used to infect *Lolium* spp. enabled loline

production in ryegrass pastures where this does not occur naturally. In this situation changes in alkaloid production have been observed whereby levels of production increased or declined or even reduced to zero. Metabolite production is not the only function that may change in a cross-species infection; the rate at which fungus is transmitted from one generation to the next via seed can also be affected.

This study has involved the isolation of *Epichloë* from *Elymus* and *Hordeum* species from the tribe Hordeeae (Triticeae) as examples of hosts from within the same tribe as the cereal grasses wheat, barley and rye. As with cross-species infections in pasture grasses, a difference in alkaloid production was observed when *Elymus* sourced *Epichloë* was used to infect rye. Additionally, a perturbation of the morphological phenotype of the novel host was observed (Results Chapter 5). *Epichloë*-induced changes in host morphology have previously been described in synthetic associations where spontaneous changes in the fungus have occurred (Simpson*, et al.*, 2012b), or where the fungus has been mutated (Tanaka*, et al.*, 2005; Johnson, 2008; Simpson & Mace, 2012c). Here however there was no evidence of changes in the fungus. Given the co-speciation of *Epichloë* and their grass hosts and the maintenance of specific associations over many generations, it may be that when there was a change in host there was a biological 'misfit' that manifested as an alteration of the host phenotype. The application of anthropogenic selection pressure to novel synthetic symbioses may select for an improved biological fit.

6.6   Desirable *Epichloë* infection-phenotypes could be selected from within outcrossing populations of rye

A key difference in *Epichloë*-induced morphological change in the target host grass could be seen between rye on the one hand and wheat and barley on the other. In rye, an obligate outcrossing species, a range of infection phenotypes was observed, whereas in wheat and barley cultivars, both selfing inbred lines, the infection phenotypes were homogenous.

The genotype of inoculated plants affected infection phenotypes. Outcrossing species such as rye presented a range of genotypes in an inoculated population whereas inbred species such as wheat and barley offered a genetically narrow host population target. Furthermore, once infection had been achieved, an outcrossing species such as rye offered the potential to bring a range of genetics over the infected maternal plant by pollination with a range of genotypes. Inbred lines such as wheat and barley offered little or no scope for producing progeny with varying genetics from the infected maternal plant.

In this study, despite the infection of barley, no seed was produced by the infected plants. Infected wheat plants were capable of producing seed and the first example of seed transmission of *Epichloë* in the seed of wheat was demonstrated, but the seed produced were compromised. The seed of AR3060-infected "Monad' wheat were light and shrivelled. Although they were viable and produced progeny plants, the progeny were either small and weak, dying prematurely or becoming endophyte-free. Rye also produced light, shrivelled seed from primary-infection plants, but these produced fertile infected progeny that produced well filled grain of similar appearance to those produced by un-infected rye plants. So, as the vegetative morphological phenotype of *Epichloë*-infected rye could be manipulated through recurrent selection, so too could aspects relating to seed phenotype including grain fill and yield parameters. This abilty to select a 'good fit'between cultivated cereal hosts and *Epichloë* is consistent with the concept of co-speciation in wild grass/ *Epichloë* symbioses.

6.7   Summary and future prospects

The aim of this thesis was to examine the possibility of deploying *Epichloë* fungal endophytes in modern cereal cultivars. The key finding was that, although cultivated cereals did not naturally host *Epichloë,* they could be artificially infected with strains isolated from related wild grasses to establish synthetic symbioses (Chapter 4). This

finding was qualified by the fact that the frequency of infection and the nature of the symbiosis was dependent on both the fungal and the host genotypes. Effects of *Epichloë* infection on the host morphological phenotype were documented. These effects varied between individuals in a population. Notably, rye, an outcrossing species, produced more functional infection phenotypes than inbreeding species such as wheat and barley. As the *Epichloë* fungus was maternally inherited into progeny seed, the opportunity existed to select germplasm from desirable phenotypes. It was demonstrated that the best phenotypes of *Epichloe*-infected rye fell within the range of phenotypes of un-infected rye and that these could form the basis for recurrent selection breeding programmes (Chapter 5). Once an *Epichloe* infection had been established in a population the germplasm could be advanced by selecting desirable symbiosis phenotypes. Screening of wild *Elymus* and *Hordeum* germplasm for *Epichloë* successfully identified strains with desirable chemotypes (Chapter 3). *Epichloe* isolated from these related wild grasses produced metabolites with proven activity against invertebrate plant pests and the symbioses with cultivated cereals that resulted from artificial infection could be selected using classical breeding methods. Useable populations of *Epichloe*-infected rye were developed within a short period (3-4 years). Wheat and barley proved to be more difficult, but the template offered by the synthetic rye/ *Epichloe* system gave insight into how such symbioses might be improved by selection of diverse primary inoculation germplasm and/or generation of genetic variability in infected populations.

A powerful method of achieving success, building on the results presented here, might be to combine breeding and inoculation right from the start by the inclusion of inoculation in cereal pre-breeding programmes. By infecting primary hybrids that lead on to Triticale cultivars or wheat addition-substitution lines, for example, rather than

taking already selected homozygous lines. This approach with self fertilising cereals is based on the better outcomes seen in rye, due to diversity. Heterozygosity is related to diversity, and primary and early generation hybrids are heterozygous and diverse, even in selfing species, so this may be the best place to start. The price to be paid is a requirement to breed varieties from the base population, which takes longer, but the potential gain is the prospect of stable associations that provide the basis for selecting several cultivars from the same base population, if it is well structured.

In summary, the findings of this thesis have provided a platform for a programme of establishing and developing *Epichloe* -infected cereal grasses with potential for improved tolerance to pests and disease along with the ability to produce greater yields in low input agricultural systems.

7 Appendices

7.1 Appendix 1 - Annotated SSR dendrogram from Card *et al* (2014).
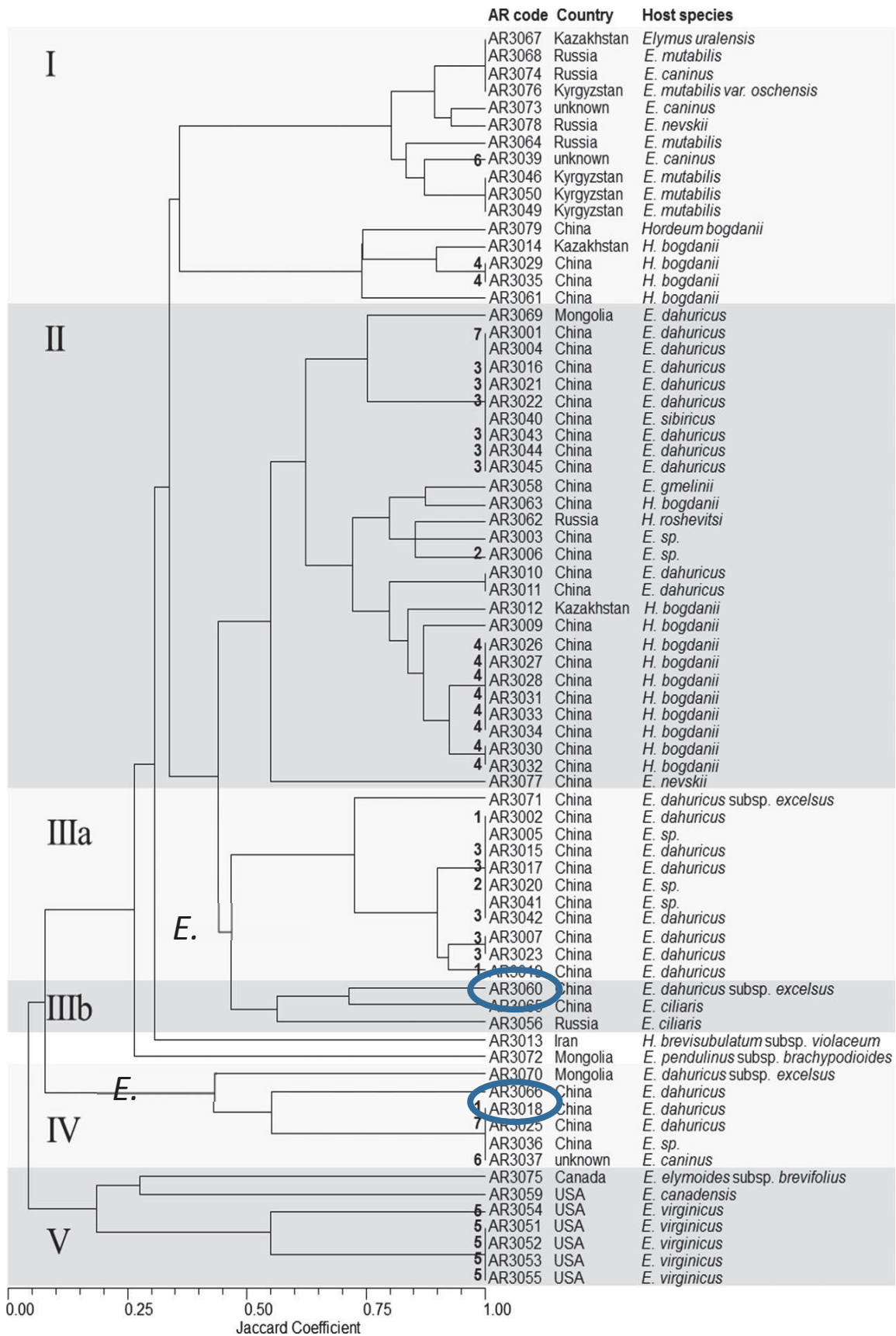
7.3 Appendix 2 – *Epichloë* strain collection

Strain	Host	Accession	Country
AR3001	*Elymus dahuricus*	BZ 2153	China
AR3002	*Elymus dahuricus*	BZ 2155	China
AR3003	*Elymus sp.*	BZ 2156	China
AR3004	*Elymus dahuricus*	BZ 2157	China
AR3005	*Elymus sp.*	BZ 2159	China
AR3006	*Elymus sp.*	BZ 2160	China
AR3007	*Elymus dahuricus*	BZ 2162	NW China
AR3008	*Hordeum bogdanii (sic)*	BZ 2191	China
AR3009	*Hordeum bogdanii*	BZ 2191	China
AR3010	*Elymus dahuricus*	BZ 2198	China
AR3011	*Elymus dahuricus*	BZ 2198	China
AR3012	*H. bogdanii*	PI 314696	
AR3013	*H. brevisubulatum*	BZ 5313	Iran
AR3014	*H. bogdanii*	PI 440414	Kazakhstan
AR3015	*Elymus dahuricus*	BZ 2162	China
AR3016	*Elymus dahuricus*	BZ 2162	China
AR3017	*Elymus dahuricus*	BZ 2162	China
AR3018	*Elymus dahuricus*	BZ 2155	China
AR3019	*Elymus dahuricus*	BZ 2155	China
AR3020	*Elymus sp.*	BZ 2160	China
AR3021	*Elymus dahuricus*	BZ 2162	China
AR3022	*Elymus dahuricus*	BZ 2162	China
AR3023	*Elymus dahuricus*	BZ 2162	China
AR3025	*Elymus dahuricus*	BZ 2153	China
AR3026	*Hordeum bogdanii*	BZ 4455	North China
AR3027	*Hordeum bogdanii*	BZ 4455	North China
AR3028	*Hordeum bogdanii*	BZ 4455	North China
AR3029	*Hordeum bogdanii*	BZ 4455	North China
AR3030	*Hordeum bogdanii*	BZ 4455	North China
AR3031	*Hordeum bogdanii*	BZ 4455	North China
AR3032	*Hordeum bogdanii*	BZ 4455	North China
AR3033	*Hordeum bogdanii*	BZ 4455	North China
AR3034	*Hordeum bogdanii*	BZ 4455	North China
AR3035	*Hordeum bogdanii*	BZ 4455	North China
AR3039	*Elymus caninus*	BZ 2679	
AR3042	*Elymus dahuricus*	BZ 2162	China
AR3043	*Elymus dahuricus*	BZ 2162	China
AR3044	*Elymus dahuricus*	BZ 2162	China
AR3045	*Elymus dahuricus*	BZ 2162	China
AR3046	*Elymus mutabilis*	BZ 4833	Kazakhstan
AR3048	*Elymus mutabilis*	BZ 4833	Kazakhstan
AR3049	*Elymus mutabilis*	BZ 4833	Kazakhstan
AR3050	*Elymus mutabilis*	BZ 4833	Kazakhstan

AR3051	*Elymus virginicus*	BZ 4820	Texas, USA
AR3052	*Elymus virginicus*	BZ 4820	Texas, USA
AR3053	*Elymus virginicus*	BZ 4820	Texas, USA
AR3054	*Elymus virginicus*	BZ 4820	Texas, USA
AR3056	*Elymus ciliaris*	BZ 4944	Russian federation
AR3057	*Elymus gmelinii*	BZ 4948	China
AR3058	*Elymus gmelinii*	BZ 4948	China
AR3059	*Elymus canadensis*	BZ 4815	Utah, USA
AR3060	*Elymus dahuricus subsp. excelsus*	BZ 4874	China
AR3061	*Hordeum bogdanii*	BZ 4969	China
AR3062	*Hordeum roshevitzi*	BZ 5030	Russian Federation
AR3063	*Hordeum bogdanii*	BZ 4968	China
AR3064	*Elymus mutabilis*	BZ 4952	Russian Federation
AR3065	*Elymus ciliaris*	BZ 4897	China
AR3066	*Elymus dahuricus*	BZ 4901	China
AR3067	*Elymus uralensis*	BZ 5083	Kazakhstan
AR3068	*Elymus mutabilis*	BZ 5339	USSR
AR3069	*Elymus dahuricus*	BZ 5466	Mongolia
AR3070	*Elymus dahuricus sbpsp. excelsus*	BZ 5473	Mongolia
AR3071	*Elymus dahuricus subsp. excelsus*	BZ 5474	China
AR3072	*Elymus pendulinus spp. brachypodioides*	BZ 5482	Mongolia
AR3073	*Elymus caninus*	BZ 5510	
AR3074	*Elymus caninus*	BZ 5564	Russian Federation
AR3076	*Elymus mutabilis var. oschensis*	BZ 5589	Estonia
AR3077	*Elymus nevskii*	BZ 5590	China
AR3079	*Hordeum bogdanii*	BZ 5602	China
AR3080	*Elymus varius*	BZ 5085	China
AR3082	*Elymus dahuricus sbpsp. excelsus*	BZ 5473	Mongolia
AR3083	*Elymus dahuricus sbpsp. excelsus*	BZ 5473	Mongolia
AR3084	*Elymus caninus*	BZ 5510	
AR3085	*Elymus nevskii*	BZ 5591	China
AR3086	*Elymus nevskii*	BZ 5591	China
AR3087	*Elymus scabrifolius*	BZ 5598	Argentina
AR3088	*Elymus scabrifolius*	BZ 5598	Argentina
AR3089	*Elymus pendulinus*	BZ 5076	Russian Federation
AR3090	Elymus sp.	BZ 5807	Georgia
AR3091	Elymus sp.	BZ 5813	Georgia
AR3092	Elymus sp.	BZ 5801	Georgia
AR3093	E. gmelinii	BZ 6902	USSR
AR3094	E. dahuricus ssp. excelsus	BZ 6923	USSR
AR3095	E. fibrosus	BZ 6935	Finland
AR3096	E. fibrosus	BZ 6937	Finland
AR3097	E. fibrosus	BZ 6937	Finland
AR3098	H. comosum [sic]	BZ 6941	Argentina
AR3099	H. comosum [sic]	BZ 6941	Argentina
AR3100	E. confusus	BZ 6464	Mongolia

AR3101	E. confusus	BZ 6464	Mongolia
AR3102	Elymus dahuricus	BZ 5470	Mongolia
AR3103	H. brevisubulatum spp. violaceum	BZ 8202	Iran
AR3104	Hordeum roshevitzii	BZ 8118	China
AR3105	Hordeum bogdanii	BZ 8119	China
AR3106	Hordeum bogdanii	BZ 8174	China
AR3108	*E. elymoides* ssp. *brevifolius*	BZ 5578	Canada
AR3109	*E. confusus*	BZ 6464	Mongolia
AR3110	*Elymus sp.*	BZ 6960	Tajikistan
AR3111	*Hordeum turkestanicum*	BZ 6970	Tajikistan
AR3112	*Elymus sp.*	BZ 6989	Tajikistan
AR3113	*Elymus sp.*	BZ 6993	Tajikistan
AR3114	*Elymus sp.*	BZ 7008	Tajikistan
AR3115	*E. mutablilis*	BZ 7291	Kazakhstan
AR3116	*E. mutablilis*	BZ 7292	Kazakhstan
AR3117	*E. mutablilis*	BZ 7310	Russian Federation
AR3118	*E. trachycaulus* ssp. *subsecundus*	BZ 8555	Canada

7.4 Appendix 3. Grass accessions screened for the presence of *Epichloë*

	Accession	Origin	Genus	Species	positive
BZ	2450	Argentina Coll	*Elymus*	*antarcticus*	0
BZ	2451	Argentina Coll	*Elymus*	*antarcticus*	0
BZ	2452	Argentina Coll	*Agropyron*	*magellanicum*	0
BZ	2453	Argentina Coll	*Elymus*	*patagonicus*	0
BZ	4408	Tas 1130. Argentina	*Elymus*	*pubiflorus*	0
BZ	4858	PI 440107 Kazakhstan	*Elymus*	*nevskii*	0
BZ	5130	GRA 2719 'Chief' Canada	*Elytrigia*	*intermedia*	0
BZ	5314	PI 440414 Kazakhstan	*Hordeum*	*bogdanii*	0
BZ	5361	PI 440420 Kazakhstan	*Hordeum*	*brevisubulatum ssp. violaceum*	0
BZ	5369	W6 26631 Kazakhstan	*Eremopyrum*	*triticeum*	0
BZ	5374	PI 565001 Kazakhstan	*Kengyilia*	*alatavica*	0
BZ	5375	PI 565002 Kazakhstan	*Kengyilia*	*batalinii*	0
BZ	5395	PI 655137 USA	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	5396	PI 440105 Kazakhstan	*Elymus*	*nevskii*	0
BZ	5404	PI 565002 Kazakhstan	*Kengyilia*	*batalinii*	0
BZ	5459	PI 655176 Argentina	*Elymus*	*andinus*	0
BZ	5479	PI 564955 Kazakhstan	*Elymus*	*mutabilis ssp. praecaespitosus*	0
BZ	5480	PI 595132 China	*Elymus*	*mutabilis ssp. praecaespitosus*	0
BZ	5488	W6 25125 Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	5521	PI 440414 Kazakhstan	*Hordeum*	*bogdanii*	0
BZ	5560	W6 13824 Argentina	*Elymus*	*antarcticus*	0
BZ	5571	PI 574531 Canada	*Elymus*	*dahuricus*	0
BZ	5578	PI 611151 Canada	*Elymus*	*elymoides ssp. brevifolius*	18
BZ	5579	W6 23470 Argentina	*Elymus*	*gayanus*	0
BZ	5580	PI 636675 Argentina	*Elymus*	*gayanus*	0
BZ	5584	PI 531617 Canada	*Elymus*	*interruptus*	1
BZ	5587	PI 387896 Canada	*Elymus*	*lanceolatus ssp. psammophilus*	0
BZ	5588	PI 531639 Russian Federation	*Elymus*	*mutabilis ssp. mutabilis*	0
BZ	5589	PI 531640 Estonia	*Elymus*	*mutabilis var. oschensis*	4
BZ	5590	W6 13133 China	*Elymus*	*nevskii*	0
BZ	5591	PI 632459 China	*Elymus*	*nevskii*	24
BZ	5592	PI 564925 Russian Federation	*Elymus*	*nevskii*	3
BZ	5597	PI 598726 Argentina	*Elymus*	*patagonicus*	0
BZ	5598	PI 331167 Argentina	*Elymus*	*scabrifolius*	0
BZ	5627	GRA 887 Canada	*Elymus*	*glaucus*	0
BZ	5628	GRA 874	*Elymus*	*nevskii*	0
BZ	5634	GRA 1203 Canada	*Elymus*	*wiegandii*	0
BZ	6427	PI 598758 JA-148 ex Kazakhstan	*Elymus*	*dahuricus*	0
BZ	6439	PI 439905 D-1880 ex Kazakhstan	*Elymus*	*caninus*	6
BZ	6441	PI 564933 AJC-241 ex Kazakhstan	*Elymus*	*fibrosus*	0
BZ	6446	PI 564905 DJ-4133 ex Kazakhstan	*Elymus*	*abolinii*	0
BZ	6451	PI 272129 ex Kazakhstan	*Elymus*	*canadensis*	0
BZ	6452	PI 236805 ex Canada	*Elymus*	*canadensis*	0
BZ	6456	PI 531545 D-3453 ex Argentina	*Elymus*	*scabrifolius*	0
BZ	6458	PI 203858 ex Argentina	*Elymus*	*agropyroides*	0
BZ	6796	PI315491	*Elymus*	*mutabilis*	0

BZ	6803	PI314631	*Elymus*	*nevskii*	0
BZ	6806	PI314622	*Elymus*	*mutabilis ssp. praecaespitosus*	0
BZ	6808	PI314620	*Elymus*	*nevskii*	0
BZ	6810	PI314618	*Elymus*	*nevskii*	0
BZ	6813	PI314614	*Elymus*	*nevskii*	0
BZ	6815	PI314611	*Elymus*	*nevskii*	0
BZ	6817	PI314209	*Elymus*	*nevskii*	0
BZ	6821	PI314204	*Elymus*	*mutabilis*	0
BZ	6824	PI314198	*Elymus*	*mutabilis ssp. praecaespitosus*	0
BZ	7291	PI 659639 Kazakhstan	*Elymus*	*mutabilis*	5
BZ	7292	PI 659641 Kazakhstan	*Elymus*	*mutabilis*	13
BZ	7293	PI 659640 Kazakhstan	*Elymus*	*mutabilis*	22
BZ	7294	PI 531638 Pakistan	*Elymus*	*mutabilis*	0
BZ	7295	PI 531636 China	*Elymus*	*mutabilis*	1
BZ	7296	PI 547375 China	*Elymus*	*mutabilis*	0
BZ	7297	PI 598549 China	*Elymus*	*mutabilis*	0
BZ	7298	PI 499589 China	*Elymus*	*mutabilis*	0
BZ	7299	PI 499449 China	*Elymus*	*mutabilis*	0
BZ	7300	PI 564953 Russian Federation	*Elymus*	*mutabilis*	0
BZ	7301	PI 564951 Russian Federation	*Elymus*	*mutabilis*	1
BZ	7302	PI 564948 Russian Federation	*Elymus*	*mutabilis*	0
BZ	7303	PI 564946 Russian Federation	*Elymus*	*mutabilis*	0
BZ	7304	PI 618746 Russian Federation	*Elymus*	*mutabilis*	1
BZ	7305	PI 628705 Russian Federation	*Elymus*	*mutabilis*	6
BZ	7306	PI 610999 Russian Federation	*Elymus*	*mutabilis*	0
BZ	7307	PI 634292 Russian Federation	*Elymus*	*mutabilis*	0
BZ	7308	PI 564950 Russian Federation	*Elymus*	*mutabilis*	0
BZ	7309	PI 610998 Russian Federation	*Elymus*	*mutabilis*	1
BZ	7310	PI 634293 Russian Federation	*Elymus*	*mutabilis*	18
BZ	7311	PI 628704 Russian Federation	*Elymus*	*mutabilis*	0
BZ	7312	PI 564952 Russian Federation	*Elymus*	*mutabilis*	0
BZ	7313	PI 628703 Russian Federation	*Elymus*	*mutabilis*	0
BZ	7363	PI 440082 Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	7364	PI 4598749 Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	7365	PI 598748 Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	7366	PI 598747 Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	7367	PI 659867 Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	7368	PI 565007 Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	7369	PI 440084 Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	7370	PI 440086 Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	7371	PI 440085Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	7372	PI 440083 Kazakhstan	*Elymus*	*repens ssp. repens*	0
BZ	7459	PI 598781 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7505	PI 598782 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7506	PI 598783 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7507	PI 598788 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7508	PI 598789 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7509	PI 598787 Kazakhstan	*Elymus*	*sibiricus*	0

BZ	7510	PI 598785 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7511	PI 598784 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7512	PI 598786 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7513	PI 598777 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7538	PI 564997 Kazakhstan	*Elymus*	*tschimganicus*	0
BZ	7539	PI 564998 Kazakhstan	*Elymus*	*tschimganicus*	0
BZ	7540	PI 547371 Kazakhstan	*Elymus*	*tschimganicus*	0
BZ	7547	PI 531567 Canada	*Elymus*	*canadensis*	0
BZ	7548	PI 452454 Canada	*Elymus*	*canadensis*	0
BZ	7580	PI 576438 Canada	*Elymus*	*dahuricus*	0
BZ	7581	PI 574531 Canada	*Elymus*	*dahuricus*	0
BZ	7612	PI 598770 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7613	PI 598761 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7614	PI 598765 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7615	PI 598763 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7616	PI 598762 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7617	PI 598768 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7618	PI 598771 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7619	PI 598760Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7620	PI 598769 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7621	PI 598772 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7622	PI 598767 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7623	PI 598764 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7624	PI 598766 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7625	PI 598759 Kazakhstan	*Elymus*	*dahuricus*	0
BZ	7773	PI 611152 Canada	*Elymus*	*elymoides ssp. brevifolius*	20
BZ	7774	PI 611151 Canada	*Elymus*	*elymoides ssp. brevifolius*	15
BZ	7775	PI 610981 Canada	*Elymus*	*elymoides ssp. brevifolius*	21
BZ	7776	PI 628688 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7777	PI 655117 United States	*Elymus*	*elymoides ssp. brevifolius*	2
BZ	7778	PI 639788 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7779	PI 655137 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7780	PI 639787 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7781	PI 639789 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7782	PI 531605 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7783	PI 659847 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7784	PI 655118 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7785	PI 659850 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7786	PI 639790 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7787	PI 659852 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7788	PI 655168 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7789	PI 655169 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7790	PI 659849 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7791	PI 659851 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7792	PI 659848 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7793	PI 655170 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7794	PI 659853 United States	*Elymus*	*elymoides ssp. brevifolius*	0
BZ	7817	PI 598779 Kazakhstan	*Elymus*	*sibiricus*	0

BZ	7818	PI 598780 Kazakhstan	*Elymus*	*sibiricus*	1
BZ	7819	PI 598778 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7820	PI 598776 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7821	PI 598773 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7822	PI 598774 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7823	PI 598775 Kazakhstan	*Elymus*	*sibiricus*	0
BZ	7828	PI 564973 Kazakhstan	*Elymus*	*sp.*	0
BZ	7829	PI 564972 Kazakhstan	*Elymus*	*sp.*	0
BZ	7855	PI 440097 Kazakhstan	*Elymus*	*trachycaulus*	0
BZ	7856	PI 440103 Kazakhstan	*Elymus*	*trachycaulus*	0
BZ	7857	PI 440098 Kazakhstan	*Elymus*	*trachycaulus*	0
BZ	7858	PI 440102 Kazakhstan	*Elymus*	*trachycaulus*	0
BZ	7859	PI 598791 Kazakhstan	*Elymus*	*trachycaulus*	0
BZ	7860	PI 531688 Kazakhstan	*Elymus*	*trachycaulus*	0
BZ	7861	PI 452446 Canada	*Elymus*	*trachycaulus*	0
BZ	7862	PI 452447 Canada	*Elymus*	*trachycaulus*	0
BZ	7863	PI 452449 Canada	*Elymus*	*trachycaulus*	0
BZ	8327	PI 655176 Argentina	*Elymus*	*andinus*	0
BZ	8344	PI 564913 Kazakhstan	*Elymus*	*caninus*	0
BZ	8379	PI 610978 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8380	PI 619489 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8381	PI 619491 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8382	PI 619553 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8383	PI 619555 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8384	PI 619561 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8385	PI 628684 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8386	PI 628685 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8387	PI 628686 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8388	PI 628687 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8389	PI 628747 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8390	PI 633741 Fish Creek USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8391	PI 655167 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8392	PI 659623 USA	*Elymus*	*elymoides ssp. elymoides*	0
BZ	8393	PI 636675 Argentina	*Elymus*	*gayanus*	0
BZ	8394	PI 611142 Kazakhstan	*Elymus*	*glaucissimus*	1
BZ	8408	PI 564928 Kazakhstan	*Elymus*	*fedtschenkoi*	0
BZ	8409	PI 564929 Kazakhstan	*Elymus*	*fedtschenkoi*	1
BZ	8414	PI 598790 Kazakhstan	*Elymus*	*fibrosus*	0
BZ	8419	PI 531617 Canada	*Elymus*	*interruptus*	0
BZ	8420	PI 547377 Canada	*Elymus*	*lanceolatus*	0
BZ	8421	PI 574515 Canada	*Elymus*	*lanceolatus*	0
BZ	8434	PI 564944 Kazakhstan	*Elymus*	*macrochaetus*	0
BZ	8435	PI 564945 Kazakhstan	*Elymus*	*macrochaetus*	1
BZ	8436	PI 618796 Kazakhstan	*Elymus*	*macrochaetus*	0
BZ	8439	PI 531633 Canada	*Elymus*	*macrourus*	0
BZ	8441	PI 531639 Russian federation	*Elymus*	*mutabilis ssp. mutabilis*	0
BZ	8442	PI 531640 Estonia	*Elymus*	*mutabilis var. oschensis*	0
BZ	8443	PI 531641 Estonia	*Elymus*	*mutabilis var. oschensis*	0

BZ	8444	PI 531642 Estonia	*Elymus*	*mutabilis var. oschensis*	0
BZ	8445	PI 564955 Kazakhstan	*Elymus*	*mutabilis ssp. praecaespitosus*	0
BZ	8446	PI 595132 China	*Elymus*	*mutabilis ssp. praecaespitosus*	0
BZ	8447	PI 440104 Kazakhstan	*Elymus*	*nevskii*	0
BZ	8448	PI 440105 Kazakhstan	*Elymus*	*nevskii*	0
BZ	8449	PI 564923 Kyrgyzstan	*Elymus*	*nevskii*	0
BZ	8450	PI 564924 Kyrgyzstan	*Elymus*	*nevskii*	0
BZ	8451	PI 564925 Russian Federation	*Elymus*	*nevskii*	1
BZ	8452	PI 632459 China	*Elymus*	*nevskii*	10
BZ	8453	PI 632570 Mongolia	*Elymus*	*nevskii*	0
BZ	8457	PI 598726 Argentina	*Elymus*	*patagonicus*	0
BZ	8473	PI 439998 Kazakhstan	*Elymus*	*repens ssp. elongatiformis*	0
BZ	8517	PI 531654 Argentina	*Elymus*	*scabriglumis*	0
BZ	8518	PI 531544 Argentina	*Elymus*	*scabrifolius*	0
BZ	8549	PI 531687 Argentina	*Elymus*	*tilcarensis*	0
BZ	8555	PI 655008 Canada	*Elymus*	*trachycaulus ssp. subsecundus*	6
BZ	8561	PI 547365 Kazakhstan	*Elymus*	*uralensis*	10
BZ	8565	PI 531548 Mexico	*Elymus*	*vaillantianus*	0
BZ	8579	PI 400988 France	*Hordelymus*	*europaeus*	0
BZ	8580	PI 442484 Belgium	*Hordelymus*	*europaeus*	0
BZ	8581	PI 531757 Austria	*Hordelymus*	*europaeus*	0
BZ	8582	PI 531758 Germany	*Hordelymus*	*europaeus*	0
BZ	8583	PI 531759 Poland	*Hordelymus*	*europaeus*	0
BZ	8584	PI 633714 Denmark	*Hordelymus*	*europaeus*	0
BZ	8585	Hordelymus	*Hordelymus*	*europaeus*	0

7.5   Appendix 4. Wheat alien addition/substitution lines inoculated with *Epichloë* strain AR3060

line	StrainID	Strain Name	Species 1	Species 2	Chromosome/Genome consitution	positive	negative
503	TACBOW0001	Le. racemosus A addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[A]	3	2
504	TACBOW0003	Le. racemosus E addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[E]	2	3
505	TACBOW0004	Le. racemosus F addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[F]	0	5
506	TACBOW0005	Le. racemosus H addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[H]	2	2
507	TACBOW0006	Le. racemosus I addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[I]	0	3
508	TACBOW0007	Le. racemosus J addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[J]	2	3
509	TACBOW0008	Le. racemosus k addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[k]	2	2
510	TACBOW0009	Le. racemosus l addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[l]	3	1
511	TACBOW0010	Le. racemosus n addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[n]	1	2
512	TACBOW0011	Le. racemosus H addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	20"+1"[H]	5	0
513	TACBOW0012	Le. racemosus 2Lr#1 addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[2Lr#1]	1	2
514	TACBOW0013	Le. racemosus 5Lr#1 addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[5Lr#1]	0	1
515	TACBOW0014	Le. racemosus 7Lr#1 addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[7Lr#1]	0	5
516	TACBOW0015	Le. racemosus 7Lr#1 addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[7Lr#1]	2	2
517	TACBOW0016	Le. racemosus ?Lr#1 addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	21"+1"[?Lr#1]	1	1
518	TACBOW0017	Le. racemosus 2Lr#1 addition	Triticum aestivum cv. Chinese Spring	Leymus racemosus	20"+1"[2Lr#1]	3	2
519	TACBOW0018	Rye 1R addition	Triticum aestivum cv. Chinese Spring	Secale cereale cv. Imperial	21"+1"[1R#1]	0	0
520	TACBOW0020	Rye 3R addition	Triticum aestivum cv. Chinese Spring	Secale cereale cv. Imperial	21"+1"[3R#1]	2	2
521	TACBOW0024	Rye 7R addition	Triticum aestivum cv. Chinese Spring	Secale cereale cv. Imperial	21"+1"[7R#1]	1	2
522	TACBOW0025	Rye 1R addition	Triticum aestivum cv. Chinese Spring	Secale cereale cv. IR130	21"+1"[1R#2]	2	3
523	TACBOW0026	Rye 1R addition	Triticum aestivum cv. Chinese Spring	Secale cereale cv. IR130	20"+1"[1R#2]	2	3
524	TACBOW0027	Rye 2R addition	Triticum aestivum cv. Chinese Spring	Secale cereale cv. IR130	21"+1"[2R#3]	0	0
525	TACBOW0028	Rye 3R addition	Triticum aestivum cv. Chinese Spring	Secale cereale cv. IR130	21"+1"[3R#3]	3	0
526	TACBOW0029	Rye 5R addition	Triticum aestivum cv. Chinese Spring	Secale cereale cv. IR130	21"+1"[5R#2]	0	1
527	TACBOW0032	Ag. intermedium 7Ai addition	Triticum aestivum cv. Vilmorin 27	Agropyron intermedium	21"+1"[7Ai#1]	1	3
528	TACBOW0034	Ag. intermedium 1Ai addition	Triticum aestivum cv. Vilmorin 27	Agropyron intermedium	21"+1"[1Ai#1]	1	1
529	TACBOW0035	Ag. intermedium 4Ai addition	Triticum aestivum cv. Vilmorin 27	Agropyron intermedium	21"+1"[4Ai#1]	0	4
530	TACBOW0036	Ag. intermedium 5Ai addition	Triticum aestivum cv. Vilmorin 27	Agropyron intermedium	21"+1"[5Ai#1]	2	2
531	TACBOW0038	Ag. elongatum 1E addition	Triticum aestivum cv. Chinese Spring	Agropyron elongatum	21"+1"[1E]	1	4

532	TACBOW0039	Ag. elongatum 2E addition	Agropyron elongatum	Triticum aestivum cv. Chinese Spring	21"+1"[2E]	0	4
533	TACBOW0040	Ag. elongatum 3E addition	Agropyron elongatum	Triticum aestivum cv. Chinese Spring	21"+1"[3E]	1	3
534	TACBOW0041	Ag. elongatum 4E addition	Agropyron elongatum	Triticum aestivum cv. Chinese Spring	21"+1"[4E]	1	3
535	TACBOW0042	Ag. elongatum 6E addition	Agropyron elongatum	Triticum aestivum cv. Chinese Spring	21"+1"[6E]	0	2
536	TACBOW0043	Ag. elongatum 5E addition	Agropyron elongatum	Triticum aestivum cv. Chinese Spring	21"+1"[5E]	1	2
537	TACBOW0044	Ag. elongatum 7E addition	Agropyron elongatum	Triticum aestivum cv. Chinese Spring	21"+1"[7E]	0	4
538	TACBOW0045	Ae. umbellulata 1U addition	Aegilops umbellulata	Triticum aestivum cv. Chinese Spring	21"+1"[1U#1]	2	2
539	TACBOW0046	Ae. umbellulata 2U addition	Aegilops umbellulata	Triticum aestivum cv. Chinese Spring	21"+1"[2U#1]	3	2
540	TACBOW0047	Ae. umbellulata 4U addition	Aegilops umbellulata	Triticum aestivum cv. Chinese Spring	21"+1"[4U#1]	1	4
541	TACBOW0048	Ae. umbellulata 5U addition	Aegilops umbellulata	Triticum aestivum cv. Chinese Spring	21"+1"[5U#1]	1	1
542	TACBOW0049	Ae. umbellulata 6U addition	Aegilops umbellulata	Triticum aestivum cv. Chinese Spring	21"+1"[6U#1]	2	2
543	TACBOW0051	Ho. chilense 1Hch substitution	Hordeum chilense	Triticum aestivum cv. Chinese Spring	20"+1"[1H^ch^]	0	5
544	TACBOW0052	Ho. chilense 2HchS addition	Hordeum chilense	Triticum aestivum cv. Chinese Spring	21"+t"[2H^ch^S]	2	1
545	TACBOW0053	Ho. chilense 4Hch addition	Hordeum chilense	Triticum aestivum cv. Chinese Spring	21"+1"[4H^ch^]	1	4
546	TACBOW0054	Ho. chilense 5Hch addition	Hordeum chilense	Triticum aestivum cv. Chinese Spring	21"+1"[5H^ch^]	2	3
547	TACBOW0055	Ho. chilense 6Hch addition	Hordeum chilense	Triticum aestivum cv. Chinese Spring	21"+1"[6H^ch^]	3	2
548	TACBOW0056	Ho. chilense 7Hch addition	Hordeum chilense	Triticum aestivum cv. Chinese Spring	21"+1"[7H^ch^]	4	1
549	TACBOW0057	Ha. villosa 1V addition	Haynaldia villosa	Triticum aestivum	21"+1"[1V#3]	1	4
550	TACBOW0059	Ha. villosa 3V addition	Haynaldia villosa	Triticum aestivum	21"+1"[3V#3]	1	4
551	TACBOW0060	Ha. villosa 4V addition	Haynaldia villosa	Triticum aestivum	21"+1"[4V#3]	0	4
552	TACBOW0061	Ha. villosa 5V addition	Haynaldia villosa	Triticum aestivum	21"+1"[5V#3]	0	2
553	TACBOW0062	Ha. villosa 6V addition	Haynaldia villosa	Triticum aestivum	21"+1"[6V#3]	0	0
554	TACBOW0064	T. aestivum-rye amphidiploid	Secale cereale cv. Imperial	Triticum aestivum cv. Chinese Spring	21"+7"	0	1
555	TACBOW0065	T. aestivum-rye amphidiploid	Secale cereale	Triticum aestivum	21"+7"	0	1
556	TACBOW0066	T. turgidum-rye amphidiploid	Secale cereale	Triticum turgidum	14"+7"	2?	0
557	TACBOW0067	T. turgidum-rye amphidiploid	Secale cereale	Triticum turgidum	14"+7"	1?	1
558	TACBOW0068	T. turgidum-Ha. villosa amphidiploid	Haynardia villosa	Triticum turgidum	14"+7"	0	4
559	TACBOW0069	T. aestivum-Ag. intermedium amphidiploid	Agropyron intermedium	Triticum aestivum	21"+7"	0	4
560	TACBOW0070	T. aestivum-Ag. intermedium amphidiploid	Agropyron intermedium	Triticum aestivum	21"+7"	0	3
561	TACBOW0123	Le. mollis A addition	Leymus mollis	Triticum aestivum cv. Chinese Spring	21"+1"[A]	0	2
562	TACBOW0124	Le. mollis G addition	Leymus mollis	Triticum aestivum cv. Chinese Spring	21"+1"[G]	2	2
563	TACBOW0125	Le. mollis H addition	Leymus mollis	Triticum aestivum cv. Chinese Spring	21"+1"[H]	2	1

#	TACBOW	Description	Background	Species	Formula	1	2
564	TACBOW0126	Ps. huashanica A addition	Triticum aestivum cv. Chinese Spring	Psathyrostachys huashanica	21"+1"[A]	1	2
565	TACBOW0127	Ps. huashanica B addition	Triticum aestivum cv. Chinese Spring	Psathyrostachys huashanica	21"+1"[B]	0	1
566	TACBOW0128	Ps. huashanica C addition	Triticum aestivum cv. Chinese Spring	Psathyrostachys huashanica	21"+1"[C]	1	0
567	TACBOW0133	Ag. intermedium B addition	Triticum aestivum cv. Chinese Spring	Agropyron intermedium	21"+1"[A#B]	0	3
568	TACBOW0136	Ag. intermedium E addition	Triticum aestivum cv. Chinese Spring	Agropyron intermedium	21"+1"[A#E]	0	2
569	TACBOW0137	Ag. intermedium F addition	Triticum aestivum cv. Chinese Spring	Agropyron intermedium	21"+1"[A#F]	0	2
570	TACBOW0138	Ag. intermedium G addition	Triticum aestivum cv. Chinese Spring	Agropyron intermedium	21"+1"[A#G]	1	4
571	TACBOW0189	Ae. caudata B addition	Triticum aestivum cv. Alcedo	Aegelops caudata	21"+1"[B#1]	1	3
572	TACBOW0190	Ae. caudata C addition	Triticum aestivum cv. Alcedo	Aegelops caudata	21"+1"[C#1]	1	0
573	TACBOW0191	Ae. caudata D addition	Triticum aestivum cv. Alcedo	Aegelops caudata	21"+1"[D#1]	0	2
574							
575	TACBOW0193	Ae. caudata E addition	Triticum aestivum cv. Alcedo	Aegelops caudata	21"+1"[E#1]	1	0
576	TACBOW0195	Ae. longissima 1S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+1"[1S^l^#2]	1	3
577	TACBOW0196	Ae. longissima 2S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+1"[2S^l^#2]	1	1
578	TACBOW0197	Ae. longissima 3S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+1"[3S^l^]	2	1
579	TACBOW0198	Ae. longissima 4/7S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+1"[4/7S^l^#2]	2	0
580	TACBOW0201	Ae. searsii 1S^s^ addition	Triticum aestivum cv. Chinese Spring	Aegelpps searsii	21"+1"[1S^s^#1]	0	0
581	TACBOW0202	Ae. searsii 2S^s^ addition	Triticum aestivum cv. Chinese Spring	Aegelpps searsii	21"+1"[2S^s^#1]	0	0
582	TACBOW0204	Ae. searsii 4S^s^ addition	Triticum aestivum cv. Chinese Spring	Aegelpps searsii	21"+1"[4S^s^#1]	1	1
583	TACBOW0205	Ae. searsii 5S^s^ addition	Triticum aestivum cv. Chinese Spring	Aegelpps searsii	21"+1"[5S^s^#1]	0	3
584	TACBOW0206	Ae. searsii 6S^s^ addition	Triticum aestivum cv. Chinese Spring	Aegelpps searsii	21"+1"[6S^s^#1]	1	2
585	TACBOW0208	Ae. longissima ?S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+1"[?S^l^]	0	4
586	TACBOW0209	Ae. longissima ?S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+1"[?S^l^]	3	1
587	TACBOW0213	Ag. elongatum 1ES addition	Triticum aestivum cv. Chinese Spring	Agropyron elongatum	21"+t"[1ES]	0	4
588	TACBOW0214	Ag. elongatum 3ES addition	Triticum aestivum cv. Chinese Spring	Agropyron elongatum	21"+t"[3ES]	2	2
589	TACBOW0215	Ag. elongatum 3EL addition	Triticum aestivum cv. Chinese Spring	Agropyron elongatum	21"+t"[3EL]	2	1
590	TACBOW0217	Ag. elongatum 6ES addition	Triticum aestivum cv. Chinese Spring	Agropyron elongatum	21"+t"[6ES]	2	3
591	TACBOW0219	Ag. elongatum 6EL addition	Triticum aestivum cv. Chinese Spring	Agropyron elongatum	21"+t"[6EL]	3	2
592	TACBOW0220	El. trachycaulus T1H^t^S-2H^t^S addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[T1H^t^S-2H^t^S]	1	1
593	TACBOW0221	El. trachycaulus T1H^t^S-3S^t^L monosomic addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21+1'[T1H^t^S-3S^t^L]	1	2
594	TACBOW0222	El. trachycaulus T1H^t^S-6H^t^L addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[T1H^t^S-6H^t^L]	3	2

595	TACBOW0224	El. trachycaulus T1S^t^L·7S^t^L addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[T1S^t^L·7S^t^L]	2	3
596	TACBOW0225	El. trachycaulus T2H^t^S·5H^t^L addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[T2H^t^S·5H^t^L]	0	1
597	TACBOW0226	El. trachycaulus T5H^t^L·5H^t^L addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[T5H^t^L·5H^t^L]	1?	0
598	TACBOW0227	El. trachycaulus T7AL·1AS-1S^t^S substitution	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	20"+1"[T7AL·1AS-1S^t^S]	0	0
599	TACBOW0228	El. trachycaulus T7AL·1AS-1S^t^S (7A) & T5DL·7AS (5D) translocation	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	19"+1"[T7AL·1AS-1S^t^S (7A)] +1"[T5DL·7AS (5D)]	0	1
600	TACBOW0229	El. trachycaulus T1H^t^S·1H^t^S addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[T1H^t^S·1H^t^S]	0	3
601	TACBOW0230	El. trachycaulus T1H^t^S·5H^t^L addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[T1H^t^S·5H^t^L]	1	1
602	TACBOW0231	El. trachycaulus T1H^t^S·7S^t^L addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[T1H^t^S·7S^t^L]	0	1
603	TACBOW0232	Ae. peregrina T3U^v^#1–? addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[T3U^v^#1–?]	1	0
604	TACBOW0233	El. trachycaulus T1H^t^S·1BL translocation	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	20"+1"[T1H^t^S·1BL]	0	3
605	TACBOW0235	rye 2R addition	Triticum aestivum cv. Chinese Spring	Secale cereale	21"+1"[2R]	3	1
606	TACBOW0236	rye 3R addition	Triticum aestivum cv. Chinese Spring	Secale cereale	21"+1"[3R]	2	0
607	TACBOW0237	rye 4R addition	Triticum aestivum cv. Chinese Spring	Secale cereale	21"+1"[4R]	0	4
608	TACBOW0239	rye 6R addition	Triticum aestivum cv. Chinese Spring	Secale cereale	21"+1"[6R]	1	1
609	TACBOW0240	rye 7R addition	Triticum aestivum cv. Chinese Spring	Secale cereale	21"+1"[7R]	0	3
610	TACBOW0242	Ae. longissima 2S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+t"[2S^l^#3]	0	0
611	TACBOW0244	Ae. longissima 4S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+1"[4S^l^#3]	2	2
612	TACBOW0245	Ae. longissima 5S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+1"[5S^l^#3]	1	0
613	TACBOW0246	Ae. longissima 6S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+1"[6S^l^#3]	1	4
614	TACBOW0247	Ae. longissima 2S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegelops longissima	21"+1"[2S^l^#4]	0	0
615	TACBOW0249	El. trachycaulus 1H^t^S addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+t"[1H^t^S]	0	4
616	TACBOW0250	El. trachycaulus 1H^t^L addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[1H^t^L]	1	2
617	TACBOW0252	El. trachycaulus 1S^t^ addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[1S^t^]	1	2
618	TACBOW0253	El. trachycaulus 5H^t^ addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[5H^t^]	0	2
619	TACBOW0254	El. trachycaulus 6H^t^ addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[6H^t^]	2	2
620	TACBOW0256	El. trachycaulus 7H^t^S addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[7H^t^S]	2	0
621	TACBOW0257	El. trachycaulus 7S^t^L addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[7S^t^L]	0	5
622	TACBOW0258	El. trachycaulus 1S^t^L addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[1S^t^L]	0	5
623	TACBOW0259	El. trachycaulus 5H^t^S addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[5H^t^S]	2	0
624	TACBOW0260	El. trachycaulus 13H^t^ addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[5H^t^L]	4	1
625	TACBOW0261	El. trachycaulus 5S^t^ addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+1"[5S^t^]	1	2
626	TACBOW0262	El. trachycaulus 5S^t^S addition	Triticum aestivum cv. Chinese Spring	Elymus trachycaulus	21"+t[5S^t^S]	1	4

627	TACBOW0264	El. ciliaris 1S^c^ addition	Triticum aestivum cv. Chinese Spring	Elymus ciliaris	21"+1"[1S^c^]	0	1
628	TACBOW0265	El. ciliaris 1^Y^ addition	Triticum aestivum cv. Chinese Spring	Elymus ciliaris	21"+1"[1Y^c^]	1	2
629	TACBOW0266	El. ciliaris 1Y^c^S addition	Triticum aestivum cv. Chinese Spring	Elymus ciliaris	21"+1"[1Y^c^S]	0	5
630	TACBOW0267	Ae. longissima ?S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegilops longissima	21"+1"[?S^l^#6]	2	2
631	TACBOW0268	Ae. longissima ?S^l^ addition	Triticum aestivum cv. Chinese Spring	Aegilops longissima	21"+1"[?S^l^#5]	0	0
632	TACBOW0270	Ae. peregrina 2S^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[2S^v^#1]	0	2
633	TACBOW0271	Ae. peregrina 3S^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[3S^v^#1]	0	0
634	TACBOW0272	Ae. peregrina 4S^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[4S^v^#1]	1	2
635	TACBOW0273	Ae. peregrina 5S^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[5S^v^#1]	1	0
636	TACBOW0274	Ae. peregrina 7S^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[7S^v^#1]	0	4
637	TACBOW0275	Ae. peregrina 1U^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[1U^v^#1]	1	2
638	TACBOW0276	Ae. peregrina 2U^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[2U^v^#1]	0	2
639	TACBOW0277	Ae. peregrina 2Uv^ addition & 2BL deletion	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	20"+1"[2U^v^#1]+1"[del 2BL]	0	2
640	TACBOW0278	Ae. peregrina 3U^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	20"+1"[3U^v^#1]	2	2
641	TACBOW0279	Ae. peregrina 4U^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[4U^v^#1]	3	1
642	TACBOW0280	Ae. peregrina 5U^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[5U^v^#1]	1	1
643	TACBOW0281	Ae. peregrina 6U^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[6U^v^#1]	0	0
644	TACBOW0282	Ae. peregrina 7U^v^ addition	Triticum aestivum cv. Chinese Spring	Aegilops peregrina	21"+1"[7U^v^#1]	2	2
645	TACBOW0283	Ae. geniculata 1M^g^ addition	Triticum aestivum cv. Chinese Spring	Aegilops geniculata	21"+1"[1M^g^#1]	1	0
646	TACBOW0284	Ae. geniculata 2M^g^ addition	Triticum aestivum cv. Chinese Spring	Aegilops geniculata	21"+1"[2M^g^#1]	0	2
647	TACBOW0286	Ae. geniculata 4M^g^ addition	Triticum aestivum cv. Chinese Spring	Aegilops geniculata	21"+1"[4M^g^#1]	0	4
648	TACBOW0287	Ae. geniculata 5M^g^ addition	Triticum aestivum cv. Chinese Spring	Aegilops geniculata	21"+1"[5M^g^#1]	0	2
649	TACBOW0288	Ae. geniculata 6M^g^ addition	Triticum aestivum cv. Chinese Spring	Aegilops geniculata	21"+1"[6M^g^#1]	1	1
650	TACBOW0290	Ae. geniculata 1U^g^ addition	Triticum aestivum cv. Chinese Spring	Aegilops geniculata	21"+1"[1U^g^#1]	0	2
651	TACBOW0291	Ae. geniculata 2U^g^ addition	Triticum aestivum cv. Chinese Spring	Aegilops geniculata	21"+1"[2U^g^#1]	2	3
652	TACBOW0292	Ae. geniculata 4U^g^ addition	Triticum aestivum cv. Chinese Spring	Aegilops geniculata	21"+1"[4U^g^#1]	0	0
653	TACBOW0293	Ae. geniculata 5U^g^ addition	Triticum aestivum cv. Chinese Spring	Aegilops geniculata	21"+1"[5U^g^#1]	1	0
654	TACBOW0295	Ae. geniculata 3U^g^ addition	Triticum aestivum cv. Chinese Spring	Aegilops geniculata	21"+1"[3U^g^#1]	2	0
655	TACBOW0296	El. ciliaris 2S^c^ addition	Triticum aestivum cv. Chinese Spring	Elymus ciliaris	21"+1"2[S^c^]	0	1
656	TACBOW0297	El. ciliaris 3S^c^ addition	Triticum aestivum cv. Chinese Spring	Elymus ciliaris	21"+1"3[S^c^]	0	2
657	TACBOW0298	El. ciliaris 1S^c^ & 5Y^c^ double addition	Triticum aestivum cv. Chinese Spring	Elymus ciliaris	21"+1"[1S^c^]+1"[5Y^c^]	0	0
658	TACBOW0299	El. ciliaris ?Y^c^ addition	Triticum aestivum cv. Chinese Spring	Elymus ciliaris	21"+1"[?Y^c^]	2	0

No.	Name		
659	Langdon X	0	0
660	Langdon X	1?	0
661	Langdon X	0	0
662	Langdon X	0	2
663	Langdon X	0	0
664	Langdon X	0	0
665	Langdon X	1?	2
666	Langdon X	0	1
667	Langdon X	0	0
668	Langdon X	0	1
669	Langdon X	0	5
670	Langdon X	0	2
671	Langdon X	1?	1
672	Langdon X	1?	2
673	Langdon X	1?	2
674	Langdon X	0	1
675	Langdon X	0	3
676	Langdon X	0	1
677	Langdon X	0	0
678	Langdon X	0	0
679	Langdon X	0	0
680	Langdon X	0	0
681	Langdon X	0	1
682	Langdon X PI 476874 (149)	1	2
683	Langdon X	0	5
684	Langdon X	0	0
685	Langdon X	0	0
686	Langdon X	0	3
687	Langdon X	0	0
688	Langdon X	0	0
689	Langdon X	0	4
690	Langdon X	0	2

691	Langdon X			0	0
692	Langdon X			0	1
693	Langdon X			0	0
694	Langdon X			0	3
695	Langdon X			0	3
696	Langdon X			0	0
697	Langdon X			0	0
698	Langdon X			0	0
699	Langdon X			0	2
700	Langdon X KU-2155 (167)			1	3
701	Langdon X KU-2156 (168)			1	0
702	Langdon X			0	2
703	Langdon X KU-2816 (170)			1	0
704	Langdon X			0	1
705	Langdon X			0	0

7.6 Appendix 5. Harvest Index data for selected spaced plant 'Rahu' rye

Endophyte	Column	Plant	height (m)	spikes	seed wt (g)	seed dry (g)	chaff wt (g)	chaff dry (g)	total biomass dry (g)	HI
AR3002	2	8	1.2	65	56	51	168	123.9	174.9	0.29
AR3002	3	1	0.8	55	5	4	88.2	72.7	76.7	0.05
AR3002	3	2	1.2	60	56	55	167.2	123.1	178.1	0.31
AR3002	6	2	0.9	65	53	46	132.2	116.7	162.7	0.28
AR3002	10	4	1.2	80	74	66	188.5	168.5	234.5	0.28
AR3002	13	7	1.1	100	139	125	216.1	196.1	321.1	0.39
AR3002	15	6	1.2	60	66	56	142.9	127.4	183.4	0.31
AR3002	17	4	0.6	70	9	7	125.2	109.7	116.7	0.06
AR3002	18	5	0.8	50	32	28	104.4	88.9	116.9	0.24
AR3002	19	2	0.8	60	39	34	104.2	84.2	118.2	0.29
AR3005	1	5	0.6	90	29	28	84.5	64.5	92.5	0.30
AR3005	2	5	0.6	55	7	5	54.2	38.7	43.7	0.11
AR3005	3	8	1.1	60	105	97	152.2	152.2	249.2	0.39
AR3005	5	7	0.7	95	14	11	109	93.5	104.5	0.11
AR3005	6	3	1.1	50	137	119	162.8	142.8	261.8	0.45
AR3005	6	7	0.6	105	37	31	124.8	104.8	135.8	0.23
AR3005	8	2	1.1	50	64	57	141.6	121.6	178.6	0.32
AR3005	9	3	1.3	55	105	95	176.5	156.5	251.5	0.38
AR3005	11	2	1.1	75	87	77	188.2	168.2	245.2	0.31
AR3005	17	1	0.7	55	47	43	80	60	103	0.42
AR3007	2	7	1.2	60	25	22	222.7	202.7	224.7	0.10
AR3007	2	10	1.1	75	23	15	125.1	109.6	124.6	0.12
AR3007	3	7	0.8	50	11	4	73.6	58.1	62.1	0.06
AR3007	4	6	1.1	70	28	20	88.3	72.8	92.8	0.22
AR3007	9	9	1.2	40	24	19	142.6	122.6	141.6	0.13
AR3007	12	1	0.8	65	30	25	100.5	85	110	0.23
AR3007	12	6	1.2	45	22	19	152	136.5	155.5	0.12
AR3007	19	2	1	45	12	9	69.3	53.8	62.8	0.14
AR3007	21	7	1.1	55	25	22	176	160.5	182.5	0.12
AR3007	21	9	1	90	20	19	123.4	107.9	126.9	0.15
AR3042	1	1	0.8	110	62	55	140.8	125.3	180.3	0.31
AR3042	5	7	1.1	55	47	41	111.6	96.1	137.1	0.30
AR3042	5	8	0.9	85	39	31	124.7	109.2	140.2	0.22
AR3042	7	2	0.6	140	42	36	113.8	98.3	134.3	0.27
AR3042	7	5	1.1	75	61	50	177.1	161.6	211.6	0.24
AR3042	7	7	1.1	90	97	88	190.3	174.8	262.8	0.33
AR3042	8	7	1.2	90	58	51	253.9	238.4	289.4	0.18
AR3042	9	2	0.8	90	52	47	143	127.5	174.5	0.27
AR3042	9	3	0.6	105	19	16	73	57.5	73.5	0.22
AR3042	9	4	1	105	108	92	238.5	223	315	0.29
AR3002T	5	7	1.2	55	52	51	147	131.5	182.5	0.28
AR3002T	7	1	0.9	50	10	8	69.5	54	62	0.13
AR3002T	9	9	0.7	40	17	15	62.6	42.6	57.6	0.26
AR3002T	10	9	0.9	55	12	9	67.2	47.2	56.2	0.16
AR3002T	11	6	1.3	45	53	49	161.5	117.4	166.4	0.29
AR3002T	13	6	1.2	60	68	61	197.2	177.2	238.2	0.26
AR3002T	14	4	1.3	70	66	64	167.3	151.8	215.8	0.30
AR3002T	22	4	1.3	45	39	31	128.5	113	144	0.22
AR3002T	25	1	0.7	80	35	32	129.6	114.1	146.1	0.22
AR3002T	25	9	1.1	45	34	32	114.5	99	131	0.24
AR3068	8	6	1.1	85	138	125	227.6	207.6	332.6	0.38
AR3068	13	5	0.7	105	22	19	99.9	84.4	103.4	0.18

AR3068	16	6	1.2	80	119	103	248.1	248.1	351.1	0.29
AR3068	16	9	1.2	140	111	103	415.2	371.1	474.1	0.22
AR3068	21	1	0.7	100	21	21	82.3	82.3	103.3	0.20
AR3068	21	5	0.7	65	15	14	59.8	44.3	58.3	0.24
AR3068	23	4	0.7	135	44	39	123.5	123.5	162.5	0.24
AR3068	25	1	0.8	155	77	70	181.6	181.6	251.6	0.28
AR3068	25	5	0.9	85	74	68	181.3	181.3	249.3	0.27
AR3068	26	6	1.1	155	193	177	324.6	309.1	486.1	0.36
AR3071	1	1	1	95	53	50	169.9	169.9	219.9	0.23
AR3074	1	6	1	90	82	72	240.4	240.4	312.4	0.23
AR3074	3	1	1.1	75	115	100	181.2	161.2	261.2	0.38
AR3074	3	8	0.8	80	25	23	104.1	104.1	127.1	0.18
AR3074	6	6	1.1	140	53	45	298.7	278.7	323.7	0.14
AR3074	10	3	1.4	55	34	28	217.8	197.8	225.8	0.12
AR3074	12	5	1.2	150	72	62	361.8	346.3	408.3	0.15
AR3074	14	1	0.7	100	16	13	103.6	103.6	116.6	0.11
AR3074	15	1	0.5	110	6	5	77.6	57.6	62.6	0.08
AR3074	16	8	0.6	135	7	6	103.4	103.4	109.4	0.05
AR3074	20	6	0.8	130	41	35	159.5	159.5	194.5	0.18
Rahu Nil	12	1			127	115	225.6	210.1	325.1	0.35
Rahu Nil	2	6			94	82	197.5	182	264	0.31
Rahu Nil	9	11			125	120	163.8	148.3	268.3	0.45
Rahu Nil	9	8			102	95	174	158.5	253.5	0.37
Rahu Nil	5	4			73	72	142.2	126.7	198.7	0.36
Rahu Nil	3	6			106	98	140.8	140.8	238.8	0.41
Rahu Nil	9	5			95	89	162.6	147.1	236.1	0.38
Rahu Nil	9	9			124	116	227.3	211.8	327.8	0.35
Rahu Nil	12	5			119	111	218	202.5	313.5	0.35
Rahu Nil	7	4			124	110	274.9	259.4	369.4	0.30

7.7 Appendix 6 Genome Designations in the Triticeae

The following is sourced from: www.herbarium.usu.edu/Triticeae/genmsymb.htm
Those attending the Second International Triticeae Consortium agreed to adopt a standard set of symbols for the individual haplomes of the Triticeae. A committee consisting of R.R.-C. Wang (Chair), R. von Bothmer, J. Dvorak, G. Fedak, I. Linde-Laursen, and M. Muramatsu met with two goals:

1) To develop a set of rules for designating haplome symbols for the Triticeae and
2) To develop a set of symbols that based on existing knowledge of the tribe.

The results of their deliberations were published in the Proceedings of the Symposium. The information presented here is based on that publication and subsequent discussions.

I have taken the liberty of proposing an additional rule. It is clearly identified as such in what follows. To my mind, preparation and citation of voucher specimens is essential if the designation of haplome symbols and publication of haplomic constitutions are to have the permanence and verifiability that is critical to scientific work.

Anyone wishing to propose changes or additions to the rules and symbols presented here should contact Dr. R.R.-C. Wang. For errors in the Web pages, contact Dr. Mary Barkworth.

Rules for Haplome Designation

Haplome symbols should be written in bold face.

Different basic haplomes in the Triticeae (with x = 7), defined as having less than 50% of complete meiotic pairing (i.e., $c < 0.5$) in a a diploid hybrid in the absence of the Ph or other pairing promoter/suppressor gene effect, should be designated with different symbols.

Single upper case letters of the Roman alphabet (A-Z) should, as far as possible, be used as symbols for the basic haplomes.

Additional basic haplomes should be designated by an upper case letter followed by a lower case letter.

The haplome designation of a polyploid taxon should be given as a combination of the symbols of its constituent basic diploid haplomes.

Unknown or unverified haplomes should be designated X followed by a lower case letter (e.g., Xu for Hordeum murinum). When a haplome has been sufficiently identified as distinct from all other established basic haplomes, it should be given its own haplome symbol.

The letter Y has previously been used to designate unknown haplomes, but it has also been extensively used to designate on the haplomes present in species of Elymus sensu lato. It is now restricted to use in Elymus sensu lato.

Modified versions of a basic haplome should be indicated by superscripts in lower case that are indicative of one of the species carrying such modified haplomes. Further modifications may be indicated by superscripted numerals.

When a previously unrecognized basic haplome is identified, a symbol should be assigned to it in accordance with these rules.

A haplome symbol may be underlined to indicate the origin of the cytoplasm of an alloploid species.

From 1996 on, the haplome symbols designations presented here should have priority over subsequent proposals.

Proposed Addition to the Rules (no action has been taken on this proposal)

Any time the haplomic constitution of a taxon is determined, a herbarium specimen shall be prepared from a mature plant of the material used. This voucher specimen must be deposited in an internationally recognized herbarium. Publication of the haplomic constitution must include the code of this herbarium and sufficient additional information to enable the specimen concerned to be identified unequivocally.

Comment: Correct identification of the material used is essential if determinations of haplomic constitution are to be of value. Deposition of voucher material ensures that determinations can be verified at a later date and that, if the taxonomy of the group involved is changed, the haplomic information can be associated with the appropriate newly recognized or modified taxa.

A useful criterion for "internationally recognized herbarium" is listing in Index Herbariorum but the most critical aspect is that it should be a herbarium whose specimens are regularly consulted by taxonomists and one that is willing to loan specimens to other herbaria on request. Personal herbaria do not meet these criteria, nor do some research station herbaria.

Haplome Symbols

The table below is based on Wang et al. (1996), but I have a) changed the generic concepts adopted, b) listed the taxa alphabetically, c) eliminated the references to previous designations, and e) added a column for listing the accession code and number of herbarium specimens that document a particular report. The reason for adding the last column is given above.

I have also started a set of files for listing all taxa in the Triticeae in which the haplomic constitution of each taxon will be listed, if it has been determined directly rather than inferred from its morphology. This set of files will be built up slowly, as time permits.

Genera listed in the table: Aegilops, Agropyron, Australopyrum, Crithopsis, Dasypyrum, Elymus (includes Elytrigia), Eremopyrum, Festucopsis, Henrardia, Heteranthelium, Hordeleymus, Hordeum, Kengyilia, Leymus, Pascopyrum, Peridictyon, Psathyrostachys, Thinopyrum (includes Lophopyrum, Trichopyrum), Triticum. [This listing is repeated at the end of the table].

Taxon	Haplomic Symbol or Constitution	Reference	Voucher Specimen
Aegilops bicornis	**Sb**		
Aegilops biuncialis	**UM**		
Aegilops caudata	**C**		
Aegilops columnaris	**UM**		
Aegilops comosa	**M**		
Aegilops crassa (4x)	**DcXc**	**Zhang et Dvorak 1992**	
Aegilops crassa (6x)	**DDcXc**	**Zhang et Dvorak 1992**	
Aegilops cylindrica	**CD**		
Aegilops juvenalis	**DcZcU**	**McNeil et al. 1994**	
Aegilops longissima	**Sl**		
Aegilops mutica	**T**	**Kimber et Tsunewaki 1988;**	
Aegilops ovata	**UM**		

Aegilops recta	**UMN, UMX**	**Kimber et Tsunewaki 1988;; Yen et Kimber 1992**	
Aegilops searsii	**Ss**		
Aegilops sharonensis	**Sl**		
Aegilops speltoides	**S. Proposal to change to B**	**Jauhar et al. 1999. Journal of heredity 90:437-445.**	
Aegilops tauschii	**D**		
Aegilops triaristata	**UMN**		
Aegilops triuncialis	**UC**	**Kimber et Tsunewaki 1988;**	
Aegilops umbellata	**U**		
Aegilops uniaristata	**N**		
Aegilops variabilis	**US USI**	**Kimber et Tsunewaki 1988; Zhang et al. 1992**	
Aegilops ventricosa	**DN**	**Kimber et Tsunewaki 1988**	
Agropyron	**P**		
Australopyrum	**W**		
Crithopsis	**K**		
Dasypyrum villosum	**V**		
Dasypyrum breviaristatum	**Vb**	**Shoji Ohta & Miki Morishita (in press)**	
Douglasdewey deweyi	**StP**		
Douglasdeweya wangyii	**StP**		
Elymus batalinii	**StPY**	**Jensen 1990**	
Elymus caucasicus	**StY**	**Jensen et Wang 1991**	
Elymus drobovii	**StHY**	**Dewey 1980**	
Elymus repens (Type species of Elytrigia)	**StStH**	**Assadi et Runemark 1994; Vershinin et al. 1994**	

Elymus scabrus	**StWY**	**Torabinejad et Mueller 1993**	
Elymus sibiricus (Type species of Elymus)	**StH**		
Elymus transhyrcanus	**StStH**	**Dewey 1972**	
Eremopyrum	**FXe**	**Sakamoto 1979; Frederiksen et Bothmer 1989**	
Festucopsis	**L**		
Henrardia	**O**		
Heteranthelium	**Q**		
Hordelymus	**XoXr**	**Bothmer et al. 1994**	
Hordeum bulbosum	**I**		
Hordeum marinum	**Xa**	**Bothmer et al. 1986**	
Hordeum murinum	**Xu**	**Bothmer et al. 1987, 1988a, 1998b**	
Hordeum vulgare	**I**		
Hordeum, other species	**H**		
Kengyilia	**StPY**	**Yen et Yang 1990**	
Leymus	**NsXm**	**Zhang et Dvorak 1991; Wang et Jensen 1994**	
Pascopyrum smithii	**StHNsXm**	**Zhang et Dvorak 1991**	
Peridictyon sanctus	**Xp**	**Seberg et al. 1991**	
Psathyrostachys	**Ns**		
Pseudoroegneria	**St**		
Pseudoroegneria deweyi	**StP**	**Jensen et al. 1992**	
Pseudoroegneria geniculata subsp. scythica	**EeSt**	**Liu et Wang 1992**	

Pseudoroegneria pertenuis	**StP**	**Wang et al. 1986; Assadi 1995**	
Secale	**R**		
Taeniatherum	**Ta**		
Thinopyrum bessarabicum	**Eb**	**Wang 1985**	
Thinopyrum caespitosum	**EeSt**	**Liu et Wang 1989, 1993b**	
Thinopyrum curvifolium	**Eb Eb**	**Liu et Wang 1993a**	
Thinopyrum distichum	**EbEe**	**Liu et Wang 1993a**	
Thinopyrum elongatum	**Ee**	**Wang 1985**	
Thinopyrum intermedium	**EeEeSt EbEeSt**	**Liu et Wang 1993b Xu et Conner 1994**	
Thinopyrum junceiforme	**EbE e**	**Liu et Wang 1992**	
Thinopyrum junceum	**EbEbEe**	**Liu et Wang 1993a**	
Thinopyrum nodosum	**EeSt**	**Liu et Wang 1993b**	
Thinopyrum sartorii	**EbE e**	**Liu et Wang 1992**	
Thinopyrum scirpeum	**EeEe**	**Liu et Wang 1993a**	
Triticum aestivum	**AuBD**		
Triticum durum	**AuB**	**Dvorak et al. 1993**	
Triticum monococcum	**Am**	**Dvorak et al. 1993**	
Triticum recta	**UMN UMX**	**Kimber et Tsunewaki 1988; Yen et Kimber 1992**	
Triticum syriacum	**DMS DcSsX**	**Kimber et Tsunewaki 1988; Zhang et Dvorak 1992**	
Triticum timopheevii	**AuG**	**Dvorak et al. 1993**	
Triticum zhukovskyi	**AmAuG**	**Dvorak et al. 1993**	

Triticum uartu	Au	Dvorak et al. 1993	

Genera listed in the table: Aegilops, Agropyron, Australopyrum, Crithopsis, Dasypyrum, Elymus (includes Elytrigia), Eremopyrum, Festucopsis, Henrardia, Heteranthelium, Hordeleymus, Hordeum, Kengyilia, Leymus, Pascopyrum, Peridictyon, Psathyrostachys, Thinopyrum (includes Lophopyrum, Trichopyrum), Triticum.

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