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Chapter 6

Epichloë Fungal Endophytes for Grassland Ecosystems

David E. Hume, Geraldine D. Ryan, Anaïs Gibert, Marjo Helander, Aghafakhr Mirlohi, and Mohammad R. Sabzalian

Abstract The *Epichloë* fungal endophytes that inhabit grasses have potentially large-scale consequences for macro- and micro-organisms and food chains in agriculture. Over 40 years of study on the benefits of symbiotic *Epichloë* fungal endophytes for host grasses, investigations have focused on the major agricultural species, tall fescue and perennial ryegrass. However, many other grass species remain to be evaluated for the effects of *Epichloë* endophytes. Animal toxicity due to accumulation of nitrogenous compounds, e.g. endophyte-derived alkaloids, particularly in areas and periods under abiotic stress, still prevent widespread application of endophyte-infected grasses in agroecosystems.

Here we review *Epichloë* endophyte-ecosystem relationships. The major points are: (1) *Epichloë* endophytes protect their host plants from vertebrate and invertebrate herbivory and allow plants to persist under water shortage, salinity, low light, mineral deficiencies and metal toxicity. Data suggests that the concentration of endophyte-derived anti-herbivore compounds increases with rising temperatures. This trend thus suggests that the strength of mutualistic interactions may increase in future climates with possible consequences for animal toxicity. (2) The benefits of endophyte infection for the host grass are context-dependent, varying with environmental conditions, grass species and cultivar, and are also highly influenced

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by interactions between both host and endophyte genetic combinations. (3) The benefits of fungal endophytes extend to neighboring grass species with respect to their protection from diseases and herbivores. (4) Novel grass-endophyte associations that produce alkaloids reducing herbivory insects, but do not produce alkaloids that are toxic to grazing vertebrates have been found. Such associations are therefore useful to control plant pests and diseases.

Keywords Agroecosystem • *Epichloë* • Endophyte • Grassland

6.1 Introduction

Cool-season grasses are widely grown in temperate regions of the world as forage for cattle, sheep and horses, as well as for turf and conservation exploitations. They may harbor internal fungal components that until recent decades were largely unknown and their significance not recognized. Fungi belonging to the *Epichloë* (Ascomycetes) genera, have both sexual and asexual (formerly *Neotyphodium* genera) forms, and confer many benefits to the grass hosts including persistence/fitness attributes; resistance/deterrence to insect feeding, drought and salinity tolerance, resistance to nematodes and fungal pathogens, and improved mineral nutrition. The production of anti-herbivore compounds by endophytes protects host plants from herbivory, however, they may have detrimental effects, causing production losses in livestock. With these complex functions, fungal endophytes can affect the economic value of forage production in natural rangelands and sown pastures, and deserve further study to explore possible applications.

Fungal endophytes have changed our past knowledge of plant physiology, phytochemistry and ecology of grasses and the factors determining plant fitness within grassland ecosystems. It is believed that after nitrogen fixing bacteria (*Rhizobium* spp.) and mycorrhizal root fungi, systemic and aerial fungal species of the genus *Epichloë* are among the most common and highly diverse microorganisms which infect many grass species of the Poaceae family.

At present, what we know about the symbiotic associations between grass and *Epichloë* endophytes is predominantly based on two model grasses: tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort. syn. *Festuca arundinacea* (Schreb.)) and perennial ryegrass (*Lolium perenne* L.) containing the obligatory fungal endophytes *Epichloë coenophiala* (formerly *Neotyphodium coenophialum*) and *Epichloë festucae* var. *lolii* (formerly *N. lolii*), respectively (Christensen et al. 1993; Leuchtman et al. 2014). In fact, the literature is strongly biased toward studies on tall fescue and perennial ryegrass while other species, especially annual and native species, of many countries have been seldom studied (Saikkonen et al. 2004, 2006; Omacini et al. 2012). Infection with *Epichloë* endophytes in these two species has been generally proven to benefit host grasses by conferring increased resistance to a wide spectra of herbivores, abiotic stress (e.g. drought, salinity) tolerance, and

enhanced growth and competitive ability of host plants (Clay and Schardl 2002). Some of these characteristics are conferred to host grasses via synthesis of fungal metabolites including the alkaloids peramine, lolines, lolitrems, and ergot alkaloids (Saikkonen et al. 2013a).

More than 100 grass species are reportedly infected with one or more fungal strains of *Epichloë* species and it is estimated that approximately 900 pooid grass species may harbor this fungal endophyte (Faeth 2002). Therefore, many previously unexamined grass species remain to be evaluated for *Epichloë* endophytes. In fact, there is a diversity of *Epichloë* strains in nature that may potentially be inoculated into new hosts. Moreover, the consequences of using common toxic strains which are found in widely grown grasses with high concentrations of toxic alkaloids versus modified and selected strains (novel endophytes) of known and newly-identified fungal symbionts in agricultural ecosystems, and possibly in strategic crop species of wheat and barley, could be significant in the future (Simpson et al. 2014).

Detecting new species and strains of fungal endophytes may also have applied value in uncovering those factors that can be manipulated to achieve more dynamic, sustainable and productive agroecosystems. Recent studies have revealed high levels of variation in the effects of endophytes on host plants, ranging from strongly positive to neutral and in some cases strongly negative (see next sections). This may suggest that these vertically transmitted endophytes and their functions are highly influenced by genetic elements including plant and *Epichloë* genotypes and their interaction, and non-genetic elements that are not currently fully known for grass-*Epichloë* endophytism.

Early research on grass endophytes was tasked with deciding whether endophyte infection was a benefit or a detriment to grasses and forage crops (Joost 1995). In fact, more than 40 years of investigation clarified that endophyte-infected plants exhibit several adaptive and resistance morphological and physiological responses to environmental stresses including insects, nematodes, drought and salinity. However, poor performance of beef cattle, sheep and horses grazing tall fescue and ryegrass infected with *Epichloë* endophytes still challenges the utilization of endophytes in grassland ecosystems. Consequently, in contrast to the idea of establishing endophyte-free pastures, other options of pasture management include minimizing the volume of toxic alkaloids ingested by domesticated animals and/or finding benign strains of *Epichloë* endophyte with minimal toxicity (Gundel et al. 2013; Young et al. 2013) to establish widely cultivated stands. Although the adoption of endophyte-free pastures may provide farmers with fast relief from animal disorders, it is not a long term economic decision. Therefore, considering the potential offered by variation revealed among endophytic isolates and their interaction with host genetic background, endophyte infection is likely to be of great utility in the management of grassland ecosystems.

A close connection between fungal endophytes and grass hosts results in mutualistic interactions between the two partners with outcomes not only for the symbiont but also sometimes the whole ecosystem that comprises many vertebrate and invertebrate organisms. Although there are many published papers on grass-*Epichloë* endophyte interactions, there are also undiscovered mechanisms with respect to

how endophyte alters grass physiology, herbivory, neighboring competitors, soils, and ultimately the whole ecosystem. In other words, the consequences of grass endophyte infection at the host level have been considered in many controlled experiments but progress has been made more slowly at the community and agroecosystem levels where benefits conferred to the host by fungal endophytes may be extended to neighboring species (Garcia Parisi et al. 2014). The aim of this review is to synthesize the literature on the present and future potential ability of *Epichloë* fungal endophyte to affect different biological components of agroecosystems and to be used for further development of grasslands around the world.

6.2 The Risk for Animal Health and Productivity in Grassland Ecosystems

Livestock consuming forage containing cool-season grasses can be affected in two ways by the presence of *Epichloë* fungal endophytes. Firstly, these endophytes can negatively impact animals consuming endophyte-infected grasses through anti-quality factors. Secondly, pasture productivity and botanical composition can be affected by endophytes, and as a result, impact on quantity and quality of forage available to livestock. Research has focused on understanding and manipulating these effects in order to achieve optimal outcomes for the sustainability, productivity and profitability of livestock industries that are dependent on grass-based forage systems (Aiken and Strickland 2013; Young et al. 2013; Johnson et al. 2013). In this section, the anti-quality aspects of *Epichloë* endophytes are reviewed. For change in pasture productivity and botanical composition, see Sects. 6.5 and 6.7.

Researchers have been able to identify a range of secondary metabolites produced when *Epichloë* endophytes are in association with cool-season grasses. The significant compounds are primarily alkaloids; peramine, lolines, indole-diterpenes and ergot alkaloids (Panaccione et al. 2014). These alkaloids convey to infected plants important economic resistance to herbivory; invertebrate pasture pests such as insects (Sect. 6.3), and grazing vertebrates such as cattle and sheep. Only the indole-diterpenes and ergot alkaloids elicit toxic responses in livestock (anti-quality factors). Their presence and concentrations can be used to predict clinical toxicity when only herbage can be analyzed and animal trials are not possible (Tor-Agbidye et al. 2001). These alkaloids can cause a range of detrimental effects to animals, both clinical and sub-clinical. These are most well-known and best described in pastures in the New World (Australia, New Zealand and USA) where endophyte-infected grass species introduced from Europe, such as ryegrass and tall fescue, dominate sown pastures.

6.2.1 Ryegrass

Perennial and long-term hybrid ryegrasses, *Lolium perenne* and *L. boucheanum* (syn. *L. hybridum*), respectively, can be infected with *Epichloë festucae* var. *lolii*. In terms of endophyte toxicity, the ryegrass-endophyte associations that became naturalized in large areas of New Zealand and southern Australia in the nineteenth century are most widely known for causing ryegrass staggers in livestock (Fig. 6.1). Ryegrass staggers is a neuromuscular disorder which occurs in late spring, summer and autumn, caused by the common toxic strain of endophyte (di Menna et al. 2012). This disorder affects a wide range of ruminants and monogastrics, including sheep, cattle, horses, donkeys, deer, goats, llamas, camels, alpacas, and rhinoceros, and has been documented to occur around the world. It has been most well studied in New Zealand where the association between ryegrass staggers and *Epichloë* endophyte was first discovered in the summer of 1980–1981 (Fletcher and Harvey



Fig. 6.1 Condition of cattle and sheep that have been grazing tall fescue (*Schedonorus arundinaceus*) or perennial ryegrass (*Lolium perenne*) pastures in the USA and New Zealand. (a) Steer that has been grazing endophyte-free tall fescue; (b) Steer that has been grazing tall fescue infected with the common toxic endophyte strain and is suffering from fescue toxicosis (Photos courtesy of John Waller, University of Tennessee, Knoxville, USA). (c) A 7 month old sheep in summer exhibiting ryegrass staggers, a neuro-muscular disorder caused by the common toxic strain of endophyte in perennial ryegrass (Photo courtesy of Lester Fletcher, AgResearch Ltd., New Zealand). (d) Bull cooling off in water due to hyperthermia as a result of grazing common toxic endophyte-infected tall fescue pasture in Arkansas (Photo courtesy of Glen Aiken, USDA-ARS, Lexington, Kentucky, USA)

1981). Pastoral agriculture in New Zealand is highly reliant on this ryegrass, and due to intense insect predation, endophyte-infected ryegrass dominates improved pastures. In some regions of New Zealand, the climate and grazing management can combine resulting in frequent and severe toxicity to livestock.

Researchers in New Zealand found that ryegrass staggers was not the only disorder caused by the common toxic endophyte strain in ryegrass (Prestidge 1993; Fletcher 1999; Fletcher et al. 1999; Watson et al. 1999) (Table 6.1). Sheep grazing common toxic endophyte typically have higher death rates (due to staggers), more fecal soiling in the breech area (termed ‘dags’) leading to higher incidence of myiasis (flystrike), increased rectal temperatures and respiration rates when under heat load, reduced feed intake, and most critically, lower live weight gains. It is one of the likely contributors to livestock failing to thrive in summer. This is likely to be linked to the common toxic endophyte not only producing tremorgenic indole-diterpenes, most notably lolitrem B which is the prime causative alkaloid of ryegrass staggers (Gallagher et al. 1981), but also ergot alkaloids which are most commonly quantified through concentration of ergovaline in herbage. The toxicity of ergot alkaloids in ryegrass-endophyte associations has parallels to the ergot alkaloid toxicity seen predominately in the USA when tall fescue is infected with its common toxic endophyte. In pasture-based dairy systems, the most substantive evidence of a toxic endophyte effect was seen in a 3-year systems experiment in New Zealand (Bluett et al. 2005). In this study, cows grazing common toxic endophyte-infected ryegrass, grown in association with white clover (*Trifolium repens*), suffered ryegrass staggers in 1 year, and over all the years, produced an average of 9 % less milk solids than cows grazing the equivalent ryegrass infected with the non-toxic AR1 endophyte which does not produce lolitrem B or ergovaline. Comparisons with endophyte-free pastures were not made as such pastures fail to survive in this region.

In Australia, livestock consuming perennial ryegrass infected with the common toxic endophyte suffer from what has been termed ‘perennial ryegrass toxicosis (PRGT)’. Livestock exhibit ryegrass staggers, heat stress, loss of productivity, and mortality to a level which can reach epidemic proportions in some years e.g. greater than 100,000 in 2002 (Reed et al. 2005; Reed et al. 2011). The phenomenon of high mortality is characteristic of Australia, and is not seen in New Zealand. While these epidemics are sporadic, ryegrass staggers in sheep can be observed every year in some regions, while subclinical losses are also likely in most years and are widespread.

The economic impact of the common toxic endophyte strain in ryegrass is substantial in New Zealand and Australia. For example, based on data from a grazing systems trial with high fertility sheep in New Zealand, Fletcher (1999) calculated common toxic endophyte-infected ryegrass had annual gross financial returns 16–18 % lower than a ryegrass system based on endophyte-free or the AR1-selected endophyte. On a national basis, PRGT in Australia in 2006 was conservatively estimated to be causing financial losses of AU \$72 million year⁻¹ (Sackett and Francis 2006), with a more recent 2012 estimate placing this at AU \$100 million year⁻¹ (Leury et al. 2014).

Table 6.1 Effects of the common toxic strain of fungal endophyte on lambs grazing only perennial ryegrass (*Lolium perenne*) in summer and autumn in Canterbury, New Zealand

Infection status of ryegrass	Ryegrass staggers score (0–5 scale, 0=nil)	Deaths due to ryegrass staggers (%)	Fecal soiling of the breech (dags) (0–5 scale, 0=nil)	Incidence of myiasis (flystrike) (%)	Liveweight gain (g head ⁻¹ day ⁻¹)	Rectal temperature (°C)	Respiration rate (breaths/minute)
Common toxic endophyte	3.1 a	5–11	1.5 a	22 a	40 b	40.6 a	99 a
No endophyte	0.4 b	0	0.4 b	2 b	110 a	40.2 b	73 b

Data is the mean of five trials over 3 years (Fletcher et al. 1999), except for deaths which is from a grazing systems trial over 3 years with ewes and lambs (Fletcher 1999). Means within a column with the same letter are not significantly different ($P < 0.05$)

Table 6.2 Threshold concentration ranges of endophyte alkaloids for the expression of clinical toxicity when fed to horses, cattle, sheep and camels

Animal species	Threshold concentration range in forage (ppm)	
	Ergovaline	Lolitrein B
Horse	0.30–0.50 ^a	0.8–1.2
Cattle	0.40–0.75	1.8–2.0
Sheep	0.50–0.80	1.8–2.5
Camel	–	≤1.1

Ergovaline causes a range of adverse effects on livestock, including fescue foot and heat stress, and is produced by both tall fescue (*Schedonorus arundinaceus*) and perennial ryegrass (*Lolium perenne*) when infected with their common toxic endophytes. Lolitrein B causes ryegrass staggers and is produced by ryegrass infected with its common toxic endophyte. For ergovaline, the threshold level is lower when animals are in cold temperatures. Compiled from di Menna et al. (1992), Tor-Agbidye et al. (2001), Aldrich-Markham et al. (2003), Fink-Gremmels (2005) and Alabdouli et al. (2014)

^aThreshold is zero for mares in late pregnancy

Ryegrass staggers in cattle has been reported in Chile (Butendieck et al. 1994), Argentina (Odriozola et al. 1993) and South Africa (Kellerman et al. 1993), all occurring on pastures sown with ryegrass seed containing high levels of common toxic endophyte. Coastal California is the only region in the USA where ryegrass staggers has been reported for cattle and sheep grazing ryegrass pastures (Galey et al. 1993), which is not surprising given the low use of this species in the USA and most US pasture cultivars are endophyte-free (Young et al. 2013). Ryegrass staggers has been recorded for endophyte-infected straw from seed production fields for turf cultivars in Oregon. This straw has also caused staggers, along with concerns over food safety, when shipped and fed to cattle and horses in Japan (Craig 2009). This toxicity in Japan led to the development of threshold alkaloid levels for toxicity of straw fed to horses, cattle and sheep (Table 6.2), and certificates for alkaloid concentrations that can be issued for straw exported to Asian countries. A limit of 2 ppm lolitrein B was set for export of straw (Young III and Silberstein 2012) based on data from the USA (Tor-Agbidye et al. 2001) and New Zealand (di Menna et al. 1992) which had established 1.8–2.5 ppm as the threshold range above which staggers could occur.

While the ryegrass endophyte is well known for its toxicity in the New World, cases in Europe of ryegrass staggers are sporadic and are small in scale (di Menna et al. 2012), and only one controlled field study has reported effects on live weight gain in sheep (Oldenburg 1998). It appears counter intuitive that Europe has low occurrence of ryegrass staggers and toxicosis, as the ryegrass endophytes causing toxicity elsewhere in the world were originally unknowingly exported from Europe by early colonizers of the New World. It is probably for this reason that endophyte studies have been reported from many European countries and have been well summarized in regular reviews (Lewis 1997, 2001; Bony and Delatour 2001; Zabalgogeaçoa and Bony 2005). Ryegrass staggers has been recorded in sheep, cattle and horses in Germany, the Netherlands, Belgium, France and the United

Kingdom. It is often associated with dry summers particularly in years of severe drought, the feeding of hay or straw, and feeding turf cultivars which are generally highly infected with common toxic endophyte. In the United Kingdom and France, ryegrass endophyte toxicoses appear to be less well-recognized, not well-documented, or on occasions misdiagnosed. Lolitrem B and ergovaline have been reported in ryegrass herbage from a range of European countries and on occasions at concentrations high enough to elicit subclinical or clinical effects in livestock if fed at a high proportion of the diet. The overall low incidence of toxicosis in Europe may be due to a range of factors. Sown pasture cultivars have no or low levels of endophyte, and while old permanent pastures may have high incidence of endophyte they are botanically diverse which would dilute endophyte toxins in the diet of grazing animals. In the drier southern regions where staggers may be expected, ryegrass is seldom the dominant species in pastures. In intensive production systems, grass is a relatively small component of the diet with high energy supplements being fed, and indoor feeding systems do not harvest the high alkaloid concentrations at the base of infected ryegrass plants. In addition, climatic conditions are only occasionally conducive (e.g. drought temporarily) to the production of high levels of toxic alkaloids.

6.2.2 *Tall Fescue*

The association of an *Epichloë* endophyte with livestock toxicity in tall fescue was first made in the southern USA in 1976 for grazing cattle (Bacon et al. 1977). In the USA, this toxicity is widespread and can be severe, being the major grass-induced toxicity in the country (Cheeke 1995). The prevalence of this toxicity can be attributed to the development and widespread use of the cultivar Kentucky 31 from the early 1940s which is infected with common toxic endophyte (Bacon 1995). In 1993, losses to the US beef industry alone were conservatively estimated at US\$ 609 million year⁻¹ (Hoveland 1993). Based on 2013 cattle prices, and a known wider effect on live weight gains, losses are now estimated to be US\$ 1.0–1.5 billion year⁻¹ (Aiken and Strickland 2013). Toxicity in livestock is characterized by three syndromes; fescue foot, bovine fat necrosis, and fescue toxicosis (summer slump or syndrome) (Strickland et al. 2009; Waller 2009), with fescue toxicosis having the greatest economic impact.

Fescue foot occurs when cattle graze endophyte-infected tall fescue in the cold temperatures of winter. As a result of a number of blood circulatory disorders, reduced peripheral blood flow results in animals developing lameness. The disorder can progress to gangrene in extremities, leading to necrosis of affected tissues with sloughing of the hooves and loss of the tips of ears and tails. When bovine fat necrosis (liptomatosis) occurs, a mass of necrotic fat builds up and occupies important space in the abdominal cavity restricting internal organs causing digestive problems and reduced reproductive capacity, all of which contribute to the symptoms of fescue toxicosis. While fescue foot and bovine fat necrosis are not generally

considered to be widespread problems, economic losses can however be significant for individual farmers when their cattle are afflicted by these syndromes.

Fescue toxicosis is most evident in the late spring and summer, when cattle fail to thrive and suffer heat stress when grazing endophyte-infected tall fescue that is otherwise of good nutritive value (Fig. 6.1). Cattle have poor summer/autumn growth rate and milk production, lowered dry matter intakes and reproduction, rough hair coat, excessive salivation, and elevated body temperature and respiration rate. Animals show an intolerance of heat, often seeking shade, forming wallows and standing in water such as dams in order to cool themselves (Fig. 6.1). The effects on live weight gains can be significant. In controlled studies, weight gains of steers and lambs have been 30–100 % greater when grazing tall fescue which is endophyte-free or infected with a non-toxic endophyte strain, compared with the equivalent tall fescue cultivar infected with the common toxic strain of endophyte (Stuedemann and Hoveland 1998; Bouton et al. 2002; Parish et al. 2003a, b). In one controlled study in Kentucky, dairy cows fed endophyte-infected tall fescue produced 20 % less milk than cows fed endophyte-free tall fescue (Strahan et al. 1987).

Growth rate of horses is reduced to a similar degree to that of cattle when ingesting endophyte-infected tall fescue (Aiken et al. 1993). However, reduced reproductive performance in mares is much greater and more important both economically and from a welfare perspective (Cross 2009). Pregnant mares grazing common toxic endophyte-infected tall fescue late in gestation suffer a range of serious symptoms, including prolonged gestations, increased abortions and foaling difficulties, high rates of agalactia (no milk), thickened and retained placentas, low pregnancy rates, and sometimes deaths during foaling. There is a high rate of stillborn foals, and live foals are weak, malformed and have low growth rates. These reproductive effects are seen widely in the eastern USA, where close to 700,000 horses, some of very high value, graze endophyte-infected tall fescue (Porter and Thompson 1992; Hoveland 1993).

The toxic symptoms seen in livestock consuming *Epichloë coenophiala*-infected tall fescue are similar to those of ergot toxicity caused by the *Claviceps* fungus that infects grass seed heads (ergots). Both fungi belong to the family Clavicipitaceae, in which ergot alkaloid production is common (Scharndl et al. 2013a). It was therefore not surprising that ergot alkaloids, and more specifically ergovaline, were determined to be the primary cause of tall fescue endophyte toxicity (Bacon 1995). The impact of varying concentrations of ergot alkaloids on productivity of cattle and sheep ingesting endophyte-infected tall fescue, and perennial ryegrass, is linear or curvilinear and appears to have no threshold below which animal live weight gains or dairy cow milk production are unaffected (Fribourg et al. 1991; Schmidt and Osborn 1993; Fletcher et al. 1999; Layton et al. 2004). In the USA, the rule of thumb is that for each 10 % increase in endophyte infection in tall fescue there is a reduced potential weight gain in yearling cattle of 45 g day⁻¹ (0.1 lb per day⁻¹). Studies with sheep grazing ryegrass in New Zealand have found a reduction in

growth rate of 3–4 g day⁻¹ in spring and up to 10 g day⁻¹ in summer-autumn for each 10 % increase in endophyte infection in ryegrass infected with an endophyte that produced ergovaline (and peramine) but no lolitrem B. Threshold concentrations have been established for expression of clinical effects, with sheep being less sensitive than cattle and horses, and thresholds being lower for ergovaline when animals are under cold temperatures and are more likely to suffer fescue foot (Tor-Agbidye et al. 2001; Craig 2009) (Table 6.2).

In Chile, Argentina and Uruguay, endophyte infection of tall fescue is widespread and can occur at high frequencies, with fescue toxicosis occurring in dairy and beef cattle (Sepulveda et al. 1996; De Battista et al. 1997; De Battista 2005). Fescue toxicosis is of greatest concern and well recognized in Uruguay and Argentina where tall fescue is the most widely sown grass species. The economic impact of toxicity can be significant, with estimated losses in Argentina in 1995 of US\$ 54 million year⁻¹ due to lower weight gains. To reduce the incidence of toxicity, current regulations in Argentina prevent the sale of seed which contains greater than or equal to 5 % endophyte infection. The greatest risk of fescue toxicosis occurs when animals graze old pastures as these can be highly infected with endophyte (Petigrosso et al. 2013), and those sown with US-imported tall fescues, mainly cultivar Kentucky 31 which also contains a toxic endophyte. The overall impact of fescue toxicosis on animal production systems is, however, considered to be somewhat less than in the USA, due to South American pastures having; greater contents of legumes which dilute the intake of endophyte toxins, a faster rate of pasture renewal, and use of locally bred or maintained cultivars that are now predominately endophyte-free.

Only a few cases of fescue toxicosis have been reported elsewhere in the world; however, when toxicity does occur it can be severe. In Australia and New Zealand, heat stress and fescue foot have been recorded in cattle, but this is confined to small areas and isolated cases for naturalized populations of tall fescue (Easton et al. 1994; Hume and Sewell 2014). In these countries, pasture cultivars of tall fescue have largely been locally bred and are free of endophyte or are infected with non-toxic endophyte strains. A single case of fescue foot in cattle grazing tall fescue has been reported in South Africa, which was most likely due to the presence of *E. coenophiala* although ergotised (*Claviceps purpurea*) seed heads were also present (Botha et al. 2004). There have been reports of fescue toxicosis in Japan when cattle have been fed tall fescue straw imported from the seed production fields of Oregon (Craig 2009). An alkaloid limit for ergovaline of 0.5 ppm has been set for tall fescue and ryegrass straw exported from Oregon (Young III and Silberstein 2012) based on clinical thresholds established by Tor-Agbidye et al. (2001) (Table 6.2). Reports of tall fescue toxicity in Europe are rare, despite reports of concentrations of ergovaline being beyond the threshold to induce clinical toxicosis (Bony and Delatour 2001). There have been a few cases of tall fescue toxicity reported in France and some suspected in Spain, while in a hay feeding experiment, Emile et al. (2000) reported that endophyte reduced weight gains of dairy heifers in France.

6.2.3 Selected Endophytes of Ryegrass and Tall Fescue

Considerable natural variation exists in *Epichloë* endophytes of perennial ryegrass and tall fescue (van Zijll de Jong et al. 2008; Ekanayake et al. 2012). This variation has been utilized to develop and commercialize cultivars infected with ‘selected’ endophytes (Thom et al. 2012; Johnson et al. 2013; Young et al. 2013). This has been possible as strains varied in production of the four classes of alkaloids, and intense research had quantified the bioactive properties of these alkaloids. The aim was to find endophytes that protected the grass plant from biotic and abiotic stresses, but had minimal clinical and subclinical toxicity to livestock. This has largely been achieved, but trade-offs between plant performance and animal performance may occur in some situations (Fletcher 2012). Despite this, there has been considerable uptake of selected endophyte technology by grassland farmers in New Zealand, Australia and USA, particularly so in New Zealand where selected endophytes in ryegrass dominate market sales (Caradus et al. 2013).

Selected endophytes have either fewer classes or lower concentrations of animal toxic alkaloids, or are completely free of animal toxic alkaloids such as lolitrem and ergot alkaloids (Fletcher 2012; Johnson et al. 2013; Young et al. 2013). These strains still produce bioprotective alkaloids which provide the host grass with resistance to invertebrate pests. For example, tall fescue infected with the selected AR542 endophyte produces peramine and N-acetylnorlooline, which have insecticidal activity, but no ergot alkaloids or lolitrem. Ryegrass infected with the Endo5 (AR5) strain produces peramine and reduced levels of the animal toxic ergovaline, but no lolitrem B thereby eliminating the risk of ryegrass staggers. Grazing studies in New Zealand, USA and Australia of ryegrass and tall fescue infected with selected strains of endophyte have shown reduced or no toxicity to sheep, cattle and horses, compared with the common toxic strains, greatly enhancing livestock health and productivity (Fletcher 1999; Bouton et al. 2002; Parish et al. 2003b; Nihsen et al. 2004; Al Rashed 2009; Beck et al. 2009; Fletcher and Sutherland 2009; Moate et al. 2012).

Exploration of this endophyte diversity and development of new grass-endophyte associations has resulted in the discovery of new bioactives. Studies of the AR37 endophyte strain in ryegrass have revealed a previously unknown group of alkaloids, epoxy-janthitrems (Tapper and Lane 2004; Fletcher and Sutherland 2009). Epoxy-janthitrems are indole-diterpenes, so it was not unexpected that sheep grazing AR37-infected ryegrass could suffer ryegrass staggers. Staggers can be as severe as that occurring on ryegrass infected with the common toxic endophyte, but for AR37, staggers are generally less severe and less frequent and other animal health performance factors are unaffected. These animal responses, combined with better agronomic performance than common toxic endophyte, have resulted in considerable uptake of AR37-infected ryegrasses in the New Zealand market with significant financial benefits to New Zealand’s pastoral industries (Caradus et al. 2013). Some unexpected consequences for animal health have occurred for some novel combinations but these have been quickly withdrawn from the market (Bourke et al. 2009; Fletcher 2012).

6.2.4 Toxicity Beyond Ryegrass and Tall Fescue

While the majority of research and economic focus has been on *Epichloë* endophyte toxicity of grasses originating from Europe, viz. perennial ryegrass and tall fescue, strong endophyte-driven toxicoses have been described for at least some grasses native to other continents of the world that are grazed in natural ecosystems (Faeth 2002). In some cases, toxicity corresponds with increased presence of the infected grass in the landscape, particularly in cases where overgrazing has occurred, and consequently important economic impacts. Other toxicosis cannot be ruled out if effects are sub-clinical and are yet to be identified.

In South Africa, cattle, horses, donkeys and, to a lesser extent sheep, consuming *Melica decumbens* ‘dronkgras’ (‘drunk grass’ in English) exhibit a drunken-like behavior, similar to ryegrass staggers (Gibbs Russell and Ellis 1982; Hoare 2014). This has been linked to infection with *Epichloë* and production of tremorgenic compounds found in other grass-endophyte associations where staggers has been reported (Miles et al. 1995a). Its presence increases in overgrazed rangelands, and its rough leaves and toxicity prevents its use in sown pastures (Hoare 2014). It is of interest that *M. decumbens* is endophyte-infected even in regions of South Africa where no staggers have been observed indicating a number of factors may be involved in the occurrence of clinical toxicity.

In grasses native to Australia and New Zealand, *Epichloë* endophytes are rare with only *Poa matthewsii* and *Echingopogon* species being identified to date as harboring this species of endophyte (Miles et al. 1998; Stewart et al. 2004). When endophyte-infected, *P. matthewsii* and *Echingopogon ovatus* produce compounds that are likely to cause toxicity to livestock, but only in Australia has a ryegrass staggers-like disorder been recorded for *Echingopogon* (Seddon and Carne 1926). The rare occurrence of clinical toxicity may be related to types of secondary metabolites produced, variation in the endophyte species that infect a particular grass species (Moon et al. 2002), and in the New Zealand landscapes due to these grasses occurring in non-grazed woodland habitats.

Achnatherum inebrians (drunken horse grass), a rangeland grass of Mongolia and northwestern China, has long been known to cause intoxication and narcosis in horses, along with donkeys, sheep, goats and cattle (Hance 1876; Miles et al. 1996). Generally most animals recover within a few days but mortality can occur in severely affected animals. *A. inebrians* has been found to be infected with *E. gan-suensis* var. *inebrians* (Li et al. 2004), and as a result, very high concentrations of ergot alkaloids have been detected and are most likely responsible for the drunken symptoms in livestock, and possibly along with stipatoxin (Dang et al. 1992; Miles et al. 1996). Incidence of toxicity is minor for animals indigenous to the region as they avoid grazing this grass, with intoxication generally occurring in animals recently imported from regions free of *A. inebrians*. The major problem for livestock farming is that *A. inebrians* is increasing in dominance due to overgrazing and other environmental factors, restricting the development of livestock farming in some regions (Li et al. 1997; Zhang et al. 2012a).

Some of the animal toxic effects of endophyte-infected *A. inebrians* in China are similar to those seen in animals grazing the closely related grass species *A. robustum* (syn. *Stipa robusta*) in south western USA. *A. robustum* is infected with the endophyte *E. funkii* and another *Epichloë* species (Faeth et al. 2006; Moon et al. 2007; Shymanovich et al. 2015), and has a common name of sleepy grass due to the narcosis of animals but other toxicities are also recorded (Jones et al. 2000). The narcotic effect is variable, which may be related to high variance in, the occurrence of endophyte in a population, *Epichloë* strain, and production of endophyte alkaloids (Jones et al. 2000; Faeth et al. 2006). In studies where ergot alkaloids have been detected, high levels have been recorded particularly of lysergic acid amide which is likely to be responsible for the narcosis in grazing animals (Petroski et al. 1992). The primary economic impact is the strong avoidance of this grass by cattle, rather than the infrequent narcosis.

In Argentina, several species of *Festuca* and *Poa* cause ‘huecu’ (‘drunk’ or ‘uncoordinated’ in English) or ‘tembaldera’ (‘tremble’ in English) toxicosis in sheep, horses and cattle (Pomilio et al. 1989), similar to symptoms seen for animals consuming *Epichloë*-infected grasses elsewhere in the world. These grasses are infected with *Epichloë tembladera* (Cabral et al. 1999), and although not proven through incisive experimentation, it is likely these ‘huecu/tembaldera’ are at least in part associated with *Epichloë*-produced indole-diterpenoid tremorgens and ergot alkaloids (Miles et al. 1995b).

6.2.5 Toxicity in Grasses Infected with Sexual *Epichloë* Endophytes

The *Epichloë*-grass toxicoses described so far are those that occur for the asexual species of *Epichloë* (formerly classified as *Neotyphodium*), fungi which are totally reliant on vertical transmission within the grass host as no sexual recombination or spread occurs (Schardl et al. 2004). Sexual *Epichloë* species, that are able to spread by horizontal transmission, produce the same classes of alkaloids as the asexual forms, including the mammalian toxins indole-diterpenes and ergot alkaloids (Lane et al. 2000; Leuchtman et al. 2000; Schardl et al. 2013b). While there is therefore a similar potential for endophyte-toxicoses to occur in grasses infected with sexual *Epichloë*, there is a tendency for this type of endophyte to have fewer and lower concentrations of known alkaloids (Siegel et al. 1990; Leuchtman et al. 2000). From an evolutionary point of view, asexual *Epichloë* are totally dependent on the fitness of the grass host for survival and propagation, so high expression of defensive alkaloids contributes strongly to the grass-endophyte mutualism (Bush et al. 1997), while sexual forms are less dependent.

There appears to be a lack of reports of livestock toxicity attributed to grazing asexual *Epichloë*-infected grasses. For example, *Festuca rubra* in the dehesa grasslands of western Spain is commonly infected to high levels with the sexual *Epichloë*

festucae (Zabalgoeazcoa et al. 1999), and at least some of these endophyte-infected plants have the potential to express ergovaline above threshold levels to be toxic to livestock (Table 6.2) (Vázquez de Aldana et al. 2003). Other ergovaline producing *Epichloë*-grass associations also occur in this ecosystem but no toxicity is reported in grazing cattle. It is postulated that the high diversity of plant species dilutes the intake of ergovaline in grazing animals in these natural ecosystems.

There are some indications that at least animal grazing behavior and preference may be affected by asexual *Epichloë* endophytes. Bazely et al. (1997) reported a positive association of grazing pressure with incidence of endophyte infection in a study with feral sheep grazing *F. rubra* on three Scottish Islands, possibly through inducible plant defense (ergovaline). A similar finding has also been reported by Koh and Hik (2007) for an asexual *Epichloë* in a subarctic alpine ecosystem for an asexual *Epichloë* infecting *Festuca altaica*.

6.2.6 *Overcoming the Economic Impact of Endophyte Toxicoses*

The animal toxicoses caused by some *Epichloë*-grass associations have stimulated considerable international research interest. Focus has primarily been on the associations and environments where both clinical and subclinical effects have been greatest and most widespread and therefore of greatest economic impact, namely the endophyte-infected sown pastures of tall fescue and ryegrass in USA, New Zealand and Australia. For many of the toxicoses reported around the world in a range of *Epichloë*-infected grasses, farmer awareness of endophyte toxicity is mostly driven by clinical effects, such as narcosis, staggers and fescue foot. Farmers may however not recognize subclinical effects or attribute them to *Epichloë* endophytes. For example, in the USA in 2004, half the farmers did not recognize that tall fescue-endophyte toxicity was a problem on their farms (Roberts and Andrae 2005) despite significant knowledge amongst research and extension personnel, and fescue toxicity costing the US cattle industry US\$ 1.0–1.5 billion year⁻¹.

Various options are available for farmers to combat the adverse effects of common toxic endophytes on animal performance and health, each option having limitations which reflect in the level of use (Aiken and Strickland 2013; Young et al. 2013). These options include, using grazing management to minimize exposure to toxic endophyte alkaloids, manipulating pasture composition to dilute alkaloids in forage, and administering treatments to livestock. Eradicating endophyte-infected tall fescue and ryegrass in cultivatable grasslands and resowing with endophyte-free cultivars removes the endophyte alkaloids that are toxic to livestock but can greatly reduce the persistence and productivity of the endophyte-free grass, which is not a tenable option in large areas of USA, New Zealand and Australia (Bouton et al. 1993; Popay et al. 1999; Hume and Sewell 2014). The option of deploying selected endophytes in pastures that can be resown is considered to be the most promising

option for capturing the endophyte-induced bioprotective and environmental stress advantages to the grass plant along with reduced or no adverse effects on livestock (Young et al. 2013). The uptake of technology has been outstanding in New Zealand for perennial ryegrass (Caradus et al. 2013).

The vertebrate-deterrent property of some endophytes has been captured in a novel application of endophytes in a non-agricultural context for the management of birds at recreational areas and airfields (Pennell and Rolston 2013). In these situations, high ergovaline-producing endophyte associations in tall fescue and ryegrass induce post digestion feedback in herbivorous birds, reducing the attractiveness of grassed areas to birds. In recreational areas, reduced grazing by large birds has decreased fecal soiling by these birds and associated risk to human health, while limiting grazing damage to turf. Where this technology has been deployed at airfields, bird numbers have potentially declined reducing the risk of bird strike on aircraft.

6.2.7 Summary of Risk for Animal Health and Productivity

The prevalence and severity of toxicity to animals consuming endophyte-infected grasses differs widely between ecosystems and farming systems, and can be dependent on weather conditions, as these affect the expression of the endophyte alkaloids. Toxicoses occur in all continents and affect a wide range of animal species. This can present a significant challenge to farmers in terms of lost productivity and animal welfare. When toxicity manifests as clinical symptoms, such as staggers, fescue foot, and narcosis, research has been relatively intense and awareness is high. Subclinical toxicity has been less well recognized but can be substantial, particularly where endophyte-infected grasses are the dominant source of feed. To varying extents, mitigation options are understood and applied, but further research is needed to understand and optimize sustainability, productivity and profitability outcomes for farmers. A challenge for researchers is to further investigate the occurrence of endophyte-infected grasses in natural and managed ecosystems, and understand their impact on animals through anti-quality factors and altering the botanical composition and productivity of pastures.

6.3 Effects of Endophyte Infection on Invertebrate Herbivory

Endophyte-infected grasses may negatively affect a wide range of herbivores from small rodents to large birds. However, most research on anti-herbivore effects of endophyte infection has focused on invertebrates, particularly insects, due to their species richness, propensity for economic damage in agroecosystems, and usefulness as ecological models. As grasses are relatively free of anti-herbivore

chemicals, endophytes and their associated mycotoxins have become a useful pest management tool for forage crops.

6.3.1 *Mechanisms of Endophyte-Associated Insect Resistance*

The defensive mutualism hypothesis was first used by Clay (1988) to describe the grass-fungal endophyte symbiosis. This framework posits that the production of defensive metabolites by fungal symbionts formed the basis of the evolution of the grass-endophyte mutualism (Clay 2009). Evolutionary models predict that vertically transmitted symbionts, such as asexual *Epichloë* endophytes, should form mutualistic interactions with host plants due to overlapping fitness (Clay 2009). It is thought that Clavicipitaceaus endophytes were derived from fungal pathogens of insects that produced biologically active secondary metabolites involved in insect pathogenicity, and later host-shifted (Spatafora et al. 2007). Empirical evidence supports the idea that endophyte-derived anti-herbivore alkaloids have a major role in enhancing host plant performance (Clay 2009); however, the majority of studies within this framework are centered around a few species of agronomic grasses (Saikkonen et al. 2010a) with relatively few studies carried out in native environments. While some studies have shown that endophyte infection can have effects on herbivores outside of alkaloid toxicity (Rasmussen et al. 2009), both artificial diet experiments (Ball et al. 1997; Yates et al. 1989) and genetic knock-out studies (Tanaka et al. 2005; Potter et al. 2008) have confirmed a causal role for endophyte-derived alkaloids in insect resistance.

Endophyte-associated resistance to invertebrate herbivores is predominantly due to the production of four groups of alkaloids: peramine, lolines, ergot alkaloids, and lolitrem (Bush et al. 1997). Peramine is a pyrrolopyrazine alkaloid and is a known insect feeding deterrent with no known activity against mammalian herbivores (Bush et al. 1997). Loline alkaloids are also potent anti-invertebrate metabolites. Depending on the insect species in question, lolines can act as overt metabolic toxins (antibiosis) or feeding deterrents (antixenosis) (Bush et al. 1997). Ergot alkaloids are also deterrent and/or toxic to an array of insect groups (Popay 2009a). While not widely associated with resistance to invertebrates, there is some limited evidence that the tremorgenic indole diterpenoid, lolitrem B, may reduce the growth and development of some invertebrates (Prestidge and Gallagher 1985). For the major agronomic grasses, lolines, peramine, and ergovaline are found in tall fescue infected with the common toxic strain of *Epichloë coenophiala*, while peramine, lolitrem B, and ergovaline are produced in *Epichloë festucae* var. *lolii*-infected perennial ryegrass (see Sect. 6.2). Some strains of *Epichloë* that contain a class of alkaloids known as janthitremes have been also shown to have species-specific and life stage-specific effects on insect performance (Tapper and Lane 2004).

Several studies have demonstrated that alkaloid concentration is linearly correlated with endophyte concentration (Rasmussen et al. 2007; Liu et al. 2011; Ryan et al. 2014a) and has been shown to depend on factors such as plant and fungal



Fig. 6.2 Endophyte-mediated resistance to insect herbivory showing endophyte-infected (*left*) and endophyte-free (*right*) meadow fescue (*Schedonorus pratensis*) plots in early spring at Lincoln, New Zealand. Damage to the endophyte-free plot is due to herbivory of roots by the larvae of grass grub *Costelytra zealandica* (Photo courtesy of Alison Popay, AgResearch Ltd., New Zealand)

genotypes (Ball et al. 2006; Rasmussen et al. 2007), soil fertility (Arechavaleta et al. 1992; Lehtonen et al. 2005a; Hunt et al. 2005; Rasmussen et al. 2007; Ryan et al. 2014a), temperature (see Sect. 6.9), CO₂ concentration (see Sect. 6.9), soil moisture (Belesky et al. 1989; Brosi et al. 2011; Kennedy and Bush 1983) and competition from other fungal symbionts (Liu et al. 2011). There is some evidence that alkaloid concentration is linearly correlated with the degree of resistance to insect herbivores (Wilkinson et al. 2000), and as such, factors that alter endophyte and alkaloid concentrations are likely to impact host plant resistance to invertebrates.

6.3.2 *Direct Effects of Endophyte Infection on Invertebrate Herbivores*

The effects of endophyte infection on insect herbivores, and tests of the defensive mutualism hypothesis, have been extensively reviewed both qualitatively (See Popay 2009a and references therein) and quantitatively (Saikkonen et al. 2010a). To date, endophyte infection from different species of *Epichloë* has been associated with resistance to more than 40 species of insect herbivores (Popay 2009b). Effects are often dramatic, as seen in Fig. 6.2, which shows the impact of endophyte infection on resistance to the grass grub *Costelytra zealandica* in meadow fescue (*Festuca*

pratensis, syn. *Schedonorus pratensis*) plots. The majority of studies have concentrated on leaf chewing and sap sucking insects (Saikkonen et al. 2010a). Endophyte-associated resistance occurs to a similar extent in these two feeding guilds, while effects on detritivorous and stem-boring insects remain inconclusive (Saikkonen et al. 2010a). Some sap sucking species, such as the aphid *Rhopalosiphum padi*, are highly sensitive to endophyte infection (Cheplick and Faeth 2009) and population abundances have been shown to decrease by up to 95 % in endophyte-infected tall fescue relative to uninfected plants (Ryan et al. 2014b). Despite the fact that fungal hyphae grow only in above-ground tissues, low concentrations of alkaloids can be found in the roots of endophyte-infected plants (Bush et al. 1993) and this has been associated with resistance to invertebrate herbivores. For example, endophyte infection has been shown to lower the performance of the parasitic plant nematodes *Pratylenchus scribneri* and *Meloidogyne maryland* in plant root tissue (Kimmons et al. 1990).

There is some evidence to suggest that endophyte-associated resistance to invertebrates can be induced, analogous to plant-derived inducible defenses found in many plant groups. Mock herbivory experiments have shown that clipping tall fescue plants infected with *Epichloë coenophiala* results in an increase in the production of loline alkaloids (Bultman et al. 2004). Induction by invertebrate herbivores may have impacts for other herbivorous insects feeding on the same plants. For example, Bultman and Ganey (1995) showed that fall armyworm larvae fed damaged endophyte-infected perennial ryegrass had lower pupal weights than those fed undamaged tissues.

The effects of endophytes on invertebrate herbivores have been variable and there are many exceptions to endophyte-associated resistance. For example, the performance of the aphid *Metopolophium festucae* on perennial ryegrass is unaffected by the presence of the common toxic strain of *Epichloë festucae* var. *lolii* (Krauss et al. 2007). While many parasitic nematodes are impacted by endophyte infection, the abundance of *Helicotylenchus pseudorobustus*, an ectoparasitic nematode, was not significantly impacted by endophyte infection in tall fescue (Kimmons et al. 1990). In a vote-counting study of the literature, Saikkonen et al. (2006) found that of 118 studies on endophyte-associated resistance to herbivores 32 % found positive, 15 % found neutral and 53 % found variable effects of endophyte on herbivore resistance. A growing body of evidence suggests that endophyte-associated resistance is stronger in agronomic grasses than natural populations (Cheplick and Faeth 2009), though natural populations have been less widely studied.

6.3.3 *Indirect Effects of Endophyte Infection on Invertebrate Herbivores*

Endophyte-associated toxicity has been shown to have broader ecosystem consequences, affecting higher trophic levels, which can feed back to indirectly impact herbivores feeding on endophyte-infected grasses. For example, endophyte

infection has been shown to cause bottom-up ecosystem effects that may alter rates of parasitism and predation on insect herbivores. Studies have shown that parasitoids of insect herbivores fed endophyte-infected plant tissue had reduced pupal mass (Bultman et al. 1997; Härrä et al. 2009) though these results did not always impact survival. It has further been demonstrated that consumption of endophyte-infected tissue by insect consumers can have consequences for hyperparasitoids (parasitoids of parasitoids). Omacini et al. (2001) found that the rate of hyperparasitism in a grass-aphid-parasitoid-hyperparasitoid model was reduced when aphids were fed endophyte-infected grasses. Studies have also demonstrated endophyte effects on insect predators. For example de Sassi et al. (2006) found that the survival of the ladybird *Coccinella septempunctata* decreased when fed on *Rhopalosiphum padi* aphids on *Epichloë*-infected perennial ryegrass. Bultman et al. (1997) found that the survival of the parasitoids *Euplectrus comstockii* and *Euplectrus plathypenae* were reduced in artificial diets containing lolines, suggesting alkaloid toxicity in non-herbivore higher trophic levels. Lehtonen et al. (2005b) demonstrated that a hemiparasitic plant acquires defending mycotoxins produced by the endophytic fungus living within their shared host grass. The uptake of defensive mycotoxins from the endophyte-infected host grass enhanced the resistance of the hemiparasitic plant to a generalist aphid herbivore. These results suggest that endophytes may have complex direct and indirect effects on several trophic levels with consequences for ecological food webs (Omacini et al. 2001).

6.3.4 Novel Grass-Fungal Associations

Endophyte infection in forage crops poses benefits, by way of enhanced resistance against insects and tolerance to abiotic stress, and simultaneous challenges, due to animal toxicity. To address this, novel grass-endophyte associations have been developed to retain those alkaloids that confer insect resistance to infected plants but do not produce those alkaloids toxic to grazing mammals (see Sect. 6.2). Such associations are produced by clearing the common toxic strain of fungus from agronomic grass cultivars and artificially inoculating with less toxic strains, or by artificially inoculating endophyte-free grasses. As described in Sect. 6.2, the *E. festucae* var. *lolii* strain 'AR1' contains peramine only, while 'AR37' produces only epoxy-janthitrems. Similarly, the *E. coenophiala* strain 'AR542' produces peramine and N-acetylnorloline only. Several other novel associations have been generated with various cultivars of perennial ryegrass (the major pasture grass in New Zealand) and tall fescue (the major pasture grass in North America). In field experiments, both AR1 and AR37 were shown to vastly reduce populations of the mealybug *Balanococcus poae* (Pennell et al. 2005) and the Argentine stem weevil *Listronotus bonariensis* (Popay and Thom 2009) relative to endophyte-free ryegrass. These reductions were similar to those observed in the common toxic strain. However, AR1 has been less successful in controlling African black beetle *Heteronychus arator*, root aphid *Aploneura lentisci* and porina *Wiseana cervinata* infestations

compared with AR37 and common toxic endophyte (Popay and Hume 2011). In AR542-infected tall fescue, mealybug infestation was reduced to levels observed in common toxic strains (Pennell and Ball 1999). The AR542 strain has also been shown to control the Argentine stem weevil and African black beetle, though the extent of reductions in herbivory were shown to be grass cultivar-specific (Popay et al. 2005).

Studies have shown that the behavior of endophytes in novel associations is dependent on host cultivar. For example, Rasmussen et al. (2007) found that the concentration of several alkaloids in AR1 and AR37 was significantly reduced in a ryegrass cultivar selectively bred to produce high levels of water-soluble carbohydrates (so-called “high sugar grasses”) relative to a “normal sugar” cultivar. Despite the clear benefits with respect to lowered insect infestation, some novel associations continued to experience animal health issues, particularly first-generation perennial ryegrass associations (Fletcher 2012). As such, the success of novel associations in agricultural systems is likely to depend on a functional understanding of metabolite profiles, toxicity responses to environmental variables, and cultivar/strain compatibility.

6.3.5 Summary of Endophyte Effects on Invertebrates

One of the most notable benefits of endophyte infection to host plants is the ability to confer resistance to herbivores. Several classes of endophyte-derived alkaloids can directly affect invertebrates through antibiosis or antixenosis, and their concentrations are dependent on the environmental context in which the host plant grows. More than 40 species of insects including sap, leaf, and root feeders, have lower performance when feeding on endophyte-infected grasses, and in some cases, alkaloid production can be induced by feeding. Endophyte infection has also been shown to cause changes in the performance of invertebrates at higher trophic levels, including predators and parasitoids of insect herbivores. Novel grass-endophyte associations have been developed for pest management and are marketed for use in agriculture. In these associations, endophyte strains are used that produce alkaloids associated with reduced insect herbivory, but do not produce those that toxic to grazing vertebrates. While research in this area is largely focused on agricultural grasses, much less is known about the effects of native grass-endophyte associations on invertebrates.

6.4 Involvement of *Epichloë* in Microbial Interactions

It has been found that plants infected with fungal endophytes produce substances that inhibit growth of some pathogens (Christensen 1996). Endophytes may counteract pathogen development indirectly through induction of plant defense

mechanisms or produce antibiotic chemicals which restrain host pathogen activity. However, compared with some other aspects of *Epichloë* endophyte effects (like resistance to insect herbivory), its influence on plant diseases have not been broadly studied. Reports on the resistance of endophyte-infected grasses to diseases and pathogens are often conflicting. Unconvincing and conflicting results may have attributed significantly to the lack of interest shown towards research concerning endophyte and pathogen interactions. Inconsistent effects of endophyte infection on host pathogens may be ascribed to different factors. The complexity of disease resistance mechanisms may be a factor (Bacon et al. 1997), and depends on both host and pathogen characteristics and their interactions, as well as their individual and collective interactions with environmental conditions (Wäli et al. 2006). Reasonably, the presence of endophyte must be considered in this complex system and in relation to the other roles of endophyte that may contribute to the ecological fitness of hosts, especially those related to abiotic stress tolerance. The large biological diversity among endophytes, and the presence of more than one *Epichloë* or other endophytic species along with *Epichloë* in a single host (as a coexisting or hybrid endophyte) may also complicate the situation, making assessment of endophyte effect on host pathogens even more difficult. Studying identical clones of a single plant genotype with and without endophyte may be a solution for finding more consistent results regarding the role of endophytes in plant-disease interactions. This approach is limited to only a few reports concerning endophyte-pathogen relations in the literature.

6.4.1 *Epichloë* Endophytes Versus Fungal Pathogens

Although *Epichloë* endophytes constitute a small proportion of the endophytic fungi connected to grass species, they are the best known and most intensively studied among the fungal endophytes discovered in grasses so far. The first report on an endophyte affecting pathogenic fungi of grasses goes back to an *in planta* study in 1983 by Shimanuki and Sato who observed that *Epichloë typhina* confers resistance to its host timothy (*Phleum pratense*) against the pathogenic fungus *Cladosporium phlei*. This suggested the possibility of mycotoxin secretion by endophyte against pathogenic fungi and triggered *in vitro* studies on the inhibiting effects of fungal endophytes. In many instances, endophyte infection depressed the growth of plant fungal pathogens (White and Cole 1985; Siegel and Latch 1991; Li et al. 2007). Similarly, liquid extracts from endophyte cultures showed inhibiting effects on a range of plant pathogenic fungi, indicating the production of antifungal compounds by the endophytes which consistently inhibit the *in vitro* growth of pathogens. For instance, three types of inhibitors isolated from a batch culture of *E. festucae*, including indole derivatives (indole-3-acetic acid and indole-3-ethanol), a sesquiterpene, and a diacetamide, were shown to have a role in disease resistance against fungal pathogens (Yue et al. 2000).

However, *in planta* effects were not completely in agreement with the *in vitro* studies. For example, Cromey and Cole (1984) reported no significant effect of the *Epichloë* endophyte on *Drechslera* leaf spot fungus, while Schmidt (1990) reported the antagonistic effect of *Epichloë* endophytes of perennial ryegrass against the same pathogen. Wheatley et al. (2001) also reported that infection of ryegrass by a leaf spot fungus (*Pyrenophora semeniperda*) was greater on endophyte-free than endophyte harboring plants for three cultivars. In another study, Clarke et al. (2006) found that infection of fine fescue by *E. festucae* enhanced resistance to dollar spot disease caused by *Sclerotinia homeocarpa*.

Gwinn and Gavin (1992) found that in a soilless medium amended with *Rhizoctonia zaeae*, survival of tall fescue seedlings increased with an increasing percentage in endophyte-infected seeds in the greenhouse condition. However, in the field, endophyte infection could not increase tall fescue resistance against blight disease caused by *Rhizoctonia solani* (Burpee and Bouton 1993). Severity of crown rust (*Puccinia coronata*) in tall fescue was reduced in endophyte-infected plants compared with endophyte-free counterparts (West et al. 1989) but there was no effect of endophyte on pathogenesis of tall fescue stem rust, *Puccinia graminis* subsp. *graminicola* (Welty et al. 1991). The effect of endophyte on crown rust infection of ryegrass was also inconsistent in Queensland, Australia (Lowe et al. 2008). In reaction to powdery mildew fungus, *Blumeria graminis*, Sabzalian et al. (2012) reported that endophyte-infected and endophyte-free tall and meadow fescues were not significantly different although endophyte-infected clones had fewer powdery mildew infected leaves and lower disease indices when compared with endophyte-free clones.

It is not clear if the same fungal-depressing compounds that are produced by endophyte *in vitro* are produced *in planta* to prevent pathogen growth and development. The other possibility is that under *in vitro* conditions where the mycelial growth of endophyte is high and no interaction exists, the endophyte produces anti-fungal chemicals in adequate amounts to depress the pathogen, but *in planta* with complex interactions present, they may not be able to produce these compounds in sufficient quantities to effectively protect the host plant from fungal diseases (Latch 2009). Certainly, there are many aspects of tripartite host-endophyte-pathogen interactions to be discovered and applied to the future breeding of grasses in their continuous battle against pathogens.

There is evidence to suggest that plant association with both *Epichloë* endophytes and mycorrhizal fungi may be regarded as mutualist–parasitist interaction and infection of grass species with *Epichloë* endophytes can suppress mycorrhizal infection. This has been shown in endophyte-infected tall fescue and ryegrass (Chu-Chou et al. 1992; Miller 2003; Omacini et al. 2006; Mack and Rudgers 2008); however, it seems that competitive interaction between the two fungal symbionts could be modified by resource supply, plant genotype and *Epichloë* endophyte strain (Liu et al. 2011).

6.4.2 *Epichloë Endophytes Versus Viruses*

Epichloë endophyte may affect host pathogenic viruses either directly, through induction of plant-derived metabolites, or indirectly by deterring pathogen-transmitting insects via chemicals produced *in planta* by the endophyte itself.

In a greenhouse experiment, Lewis and Day (1993) found that when ryegrass plants were infected with ryegrass mosaic virus and barley yellow dwarf virus (BYDV), cumulative total biomass was greater for *Epichloë*-infected plants than endophyte-free versions. The authors concluded that this may show reduction in the effect of virus infection when the plant is also infected by *Epichloë* endophyte. Comparing a common toxic and a non-toxic fungal endophyte infecting tall fescue, Rúa et al. (2013) showed that endophyte increased overall plant biomass, reduced the negative effect of virus infection on the root fraction, and stimulated tiller production, possibly by increasing plant stimulating regulators compared with endophyte-free plants. This may enhance host tolerance to viral infection by reducing viral titre in plant tissues. Also, plants infected with the common toxic endophyte supported less aphid production, abundance of adult aphids and total number of aphids and thereby enhanced host tolerance to viral infection.

Compared with the direct effect of endophytes on pathogenic viruses, the indirect effects have been more widely investigated. An indirect effect of *Epichloë* endophytes on pathogenic viruses is through deterrence or detrimental influence on virus-transmitting insects, especially where endophyte infection interferes with survival of aphids (Mahmood et al. 1993; Rúa et al. 2013). *Epichloë* endophytes are well known to produce different classes of biologically active chemicals including various lolines and peramine, both harmful and effective in reducing aphid population size, feeding time and the resulting damage to the host plant (Schardl et al. 2004).

The importance of endophytes in deterring aphids from infected hosts was recognized as early as 1985 by Johnson et al. who reported that loline alkaloids produced by the endophyte inside tall fescue can deter *Rhopalosiphum padi* aphids, the vector for barley yellow dwarf virus which is one of the most important viruses infecting small grains. The virus is transferred by aphids, which may be deterred by endophyte-derived alkaloids within the plant. However, similar to interactions between endophyte and fungal pathogens, there are also some inconsistent results on endophyte-virus interactions. In roadside tall fescue in Tasmania, neither the incidence of barley yellow dwarf virus nor the occurrence of the virus vector, *R. padi* differed between endophyte-infected and endophyte-free plants; though, some endophyte-infected tall fescue plants were more resistant to BYDV than the others (Guy and Davis 2002). Also, no effects of endophyte infection on incidence of barley yellow dwarf virus in ryegrass (Hesse and Latch 1999) or on growth response of ryegrass plants infected with virus (either BYDV or ryegrass mosaic virus) (Lewis 2004) have been found. In contrast, Lehtonen et al. (2006) showed that when aphid vectors were released on endophyte-infected and endophyte-free plants, the number of aphids and the percentage of barley yellow dwarf virus infection were lower in endophyte-

infected plants compared with endophyte-free counterparts. They also concluded that the low infection rate of barley yellow dwarf virus in endophyte containing plants may protect neighboring plants from the virus as a result of lower population sizes of aphids. This may have applications in agroecosystems by sowing endophyte-containing plants next to cereals defenseless against heavy infection by barley yellow dwarf virus, and thereby reducing grain yield losses caused by the virus.

6.4.3 *Epichloë Endophytes Versus Bacterial Populations*

The effects of *Epichloë* endophyte on plant tolerance to diseases caused by bacteria have not been assessed to the same extent as those of pathogenic fungi. It is remarkable that this aspect of *Epichloë* endophytes have so far been neglected in the research, particularly at the level of pastoral and grassland systems where endophyte-infected plants could affect neighboring species and their associated pathogens. This is despite reports that mycorrhizal plants have increased their host's resistance to virulent bacterial pathogens (Liu et al. 2007).

In soil, it seems that endophyte infection of grasses may alter micro-environmental conditions so that decomposition is slower for endophyte-infected fescue litter than for endophyte-free one (Lemons et al. 2005), though this may not be due to the change in bacterial activity (Casas et al. 2011). Recent research also showed that several bacterial species recovered from tall fescue could use N-formyl loline as a carbon and nitrogen source (Roberts and Lindow 2014) meaning that bacterial populations may be resistant to alkaloids and/or other *Epichloë* fungal metabolites. The lack of information suggests that future research at *in vitro* and *in planta* levels are needed to elucidate how bacterial pathogens could be influenced by fungal endophyte of grasses.

6.4.4 *Summary of Endophyte Involvement in Microbial Interactions*

Epichloë fungal endophytes produce some antimicrobial compounds which may inhibit the *in vitro* growth of pathogens. However, the dynamics of pathogenicity involve complex interactions between variables including environmental condition, plant genotype, *Epichloë* endophyte and pathogen strains, and as such *in planta* observations do not consistently point to prevention of pathogen growth and development. This complexity may suppress secretion of sufficient quantities of antibiotic compounds and increase resistance of pathogens to *Epichloë* fungal metabolites. Nevertheless, fungal endophytes may enhance resistance to pathogens indirectly through deterring insect vectors of plant pathogens, and by also improving the general health condition of plants via enhanced growth, improved nutritional status and abiotic stress tolerance.

6.5 *Epichloë* Endophyte Affects the Competitive Ability and Persistence of Host Plants

Inter- and intraspecific plant competition is one of the major driving forces of natural and agricultural grassland communities. Because systemic grass endophytes have been found to increase plant tolerance in stressful abiotic environments and affect all types of plant-plant, plant-herbivore and plant pathogen interactions (Clay and Holah 1999; Saikkonen et al. 2006; Wäli et al. 2006), they may potentially shape both natural grassland communities and agroecosystems. Here, the known features of plant-plant communications and competitive ability of grasses affected by *Epichloë* endophyte are briefly reviewed.

6.5.1 *Competitive Ability Depends on Environmental Conditions*

The outcome of competition is conditional and depends on both biotic and abiotic factors (Callaway et al. 1996), which are potentially affected by endophyte-mediated interactions in grasses. In various studies, endophyte-infected grasses showed increased growth vigor, and become stronger competitors compared with uninfected counterparts and co-occurring plant species (Clay and Holah 1999; Saikkonen 2000; Rudgers et al. 2005; Saikkonen et al. 2006). It is believed that, their superiority is a result of increased fitness under harsh environments such as drought and flooding, and increased resistance to invertebrate and vertebrate herbivores, seed predators and plant pathogens (Clay and Schardl 2002; Saikkonen et al. 2006). Many effects of the grass-endophyte partnership, which has often been regarded as a mutualistic interaction in many cool-season grasses, are directly influenced by the endophytic partner. As a result of this capability, endophyte-infected grasses have even been claimed to threaten native plant diversity and associated food webs (Clay and Holah 1999).

Nutrient availability of soils plays a critical role in strengthening endophyte-plant associations because in low nutrient conditions, the costs associated with harboring the systemic endophyte may override its benefits to the host grass. At the same time, the majority of studies on endophyte-mediated competitive ability and grass persistence have used agricultural settings with high nutrient availability. However, in experiments comparing low and high fertilized soils, the competitive ability of endophyte-infected plants was shown to be dependent on nutrient availability (Dirihan et al. 2014). Dirihan et al. (2014) reported that during the early phase of establishment, neither meadow fescue nor tall fescue gained instant endophyte-promoted competitive advantage over red clover when sown together. In nutrient limited soils, plant competition or the cost of endophyte infection even decreased the yield of meadow fescue. There are several reports indicating that in agricultural soils with high nutrient availability, the endophyte infection can increase the performance of the host grass but the positive effects still depend on the host species and the species composition of the grassland (Table 6.3).

Table 6.3 Effects of *Epichloë*-infected grasses on competitive neighboring species

Grass infected by <i>Epichloë</i> endophyte	Endophyte species	Competing species	Competitive effects	Reference
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	<i>Trifolium pratense</i> <i>T. repens</i> <i>Medicago sativa</i>	Negative and neutral effects on legume yield	Hoveland et al. (1999)
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	<i>Trifolium pratense</i>	Positive and negative effects on yield	Malinowski et al. (1999)
<i>Festuca arizonica</i>	<i>Epichloë</i> sp.	<i>F. arizonica</i>	Higher biomass in uninfected plants	Faeth et al. (2004)
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	Native prairie plants	Increased invasiveness to high diversity communities	Rudgers et al. (2005)
<i>Schedonorus pratensis</i>	<i>Epichloë uncinata</i>	<i>Lolium perenne</i> <i>Trifolium repens</i> <i>Dactylis glomerata</i>	Increased persistence and biomass of infected <i>S. pratensis</i>	Takai et al. (2010)
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	Invading weeds	Plant and endophyte genotype affect plant composition	Rudgers et al. (2010)
<i>Festuca rubra</i>	<i>Epichloë festucae</i>	<i>Trifolium pratense</i> , <i>T. repens</i> , <i>Lotus corniculatus</i> , <i>Plantago lanceolata</i>	Negative allelopathic effects on seed germination and radicle growth of competing plants	Vazquez de Aldana et al. (2011)
<i>Schedonorus pratensis</i>	<i>Epichloë uncinata</i>	Invading weeds	Infected plants hindered weed invasion	Saikkonen et al. (2013b)
<i>Festuca rubra</i>	<i>Epichloë festucae</i>	<i>Trifolium pratense</i>	Decrease in shoot and root biomass of <i>T. pratense</i>	Vazquez de Aldana et al. (2013b)
<i>Lolium perenne</i> <i>Schedonorus arundinaceus</i> <i>Schedonorus pratensis</i>	<i>Epichloë festucae</i> var <i>lolii</i> <i>Epichloë coenophiala</i> <i>Epichloë uncinata</i>	<i>Lolium perenne</i> <i>Bromus catharticus</i> <i>Trifolium repens</i>	Positive and negative growth in <i>Trifolium</i> , depending on fungal and grass species	Cripps et al. (2013)

(continued)

Table 6.3 (continued)

Grass infected by <i>Epichloë</i> endophyte	Endophyte species	Competing species	Competitive effects	Reference
<i>Schedonorus arundinaceus</i> <i>Schedonorus pratensis</i>	<i>Epichloë coenophiala</i> <i>Epichloë uncinata</i>	<i>Trifolium pratense</i> <i>Schedonorus arundinaceus</i> <i>Schedonorus pratensis</i>	Negative effects on biomass of infected <i>S. pratensis</i> in low nutrient soils	Dirihan et al. (2014)

On the other hand, on many occasions the effects of endophyte infection on the competitive ability of grasses turn out to be neutral or negative. In fact, the outcome is dependent on the abiotic and biotic environmental factors, grass species, grass and fungal genotype, and genetic combination of the plant and the fungus (Saikkonen 2000; Faeth et al. 2004; Rudgers et al. 2010; Saikkonen et al. 2006, 2010b). Several studies have demonstrated that endophyte-promoted competitive superiority of host plants may be most pronounced in selectively bred grass cultivars growing in nutrient rich agroecosystems and in environments where grazing pressure is high (Cheplick and Faeth 2009; Saikkonen et al. 2004, 2006, 2010a). However, in natural grasslands and other more variable environments, the grass-endophyte symbiosis may form a continuation of interactions from mutualism to antagonism depending on the grass species and/or genotype and environmental conditions. Studies using native grass species to study competitive abilities of endophyte-infected and uninfected grasses have shown that in some environments, endophyte-free grasses outperform their infected counterparts (Faeth et al. 2004).

6.5.2 *Species Diversity in Ecosystem as Affected by Endophyte Infection*

Recent evidence suggests that endophytic fungi can strongly affect grassland plant community composition and productivity both in natural and agroecosystems (Clay and Holah 1999; Rudgers et al. 2010; Saikkonen et al. 2013b). Increased plant growth, reproduction and resistance to various biotic and abiotic factors give infected grasses the ability to invade and compete in fields and grasslands.

The competitive ability of endophyte-infected tall fescue cultivar ‘Kentucky 31’ is observed to be high compared with uninfected conspecifics of the same cultivar. After a 4-year field study, Clay and Holah (1999) suggested that endophyte-infected plants were reducing species diversity in successional fields by outcompeting native plant species. The competitive superiority of endophyte-infected Kentucky 31 may, however, be more related to a lack of genetic diversity of the cultivar in the new environment and the systemic endophyte, rather than a common phenomenon of the

grass species (Saikkonen 2000). This is because tall fescue is a species originating from Europe, where it has not shown competitive superiority but is rather occurring in competition-free environments in river banks, sea shores and waste lands (Saari et al. 2010). Noteworthy is that these native European tall fescue populations are highly infected with systemic endophytes (Saari et al. 2010).

In a northern European agronomic field, a long-term study using endophyte-infected and endophyte-free meadow fescue cultivar ‘Kasper’ monocultures demonstrated that endophyte infection promoted competitive dominance of the grass and retarded weed invasion to the field (Saikkonen et al. 2013b). The maintenance of a high frequency of endophyte infections and superior productivity of infected grasses was shown to be a result of higher survival, growth and reproduction of the infected plants and not mediated by endophyte-origin substances inhibiting seed germination of endophyte-free plants. After 6 years, the percentage cover and biomass of uninfected meadow fescue monocultures was significantly reduced compared with infected meadow fescue monocultures and the species richness, percentage cover and biomass of the weed species were markedly higher.

6.5.3 *Allelopathic Interaction between Infected and Non-infected Species*

It has been suggested that the competitive superiority of endophyte-infected grasses, in addition to enhanced growth and reproduction, is based on allelopathy i.e. endophyte-mediated chemical effects on the abiotic and biotic properties of soils, which in turn may influence the composition and persistence of plant communities. The effect of plant-soil feedback has important implications both in natural and managed ecosystems. The allelopathic potential of endophyte-infected grasses has recently received increasing attention, with researchers aiming to explain mechanisms behind observed competitive superiority of endophyte-infected grasses (Antunes et al. 2008; Cripps et al. 2013). Allelochemical by-products of infected grasses, which are mostly alkaloids, could enter the soil through plant roots or from decomposing plant material (Siegrist et al. 2010). Such endophyte-derived compounds are suggested to directly inhibit the growth of other plants or suppress mutualistic microbes e.g. mycorrhizal fungi of neighboring plants (Antunes et al. 2008). Experiments using species mixtures to study allelopathic effects of endophytes have shown both negative and positive effects of endophyte on plant performance (Table 6.3). Cripps et al. (2013) found that the outcome of these allelopathic effects via soil was conditional on both endophyte-infected grass species and competing plant species studied. The negative conditioning effects of endophytes could be also due to reduced nutrient availability, altered microbial composition, endophyte-derived alkaloids in the soils and/or alterations to other root exudates (McNear and McCulley 2012).

6.5.4 Weed Management Using Endophyte-Infected Grasses

Overall benefits associated with endophytes in managed fields are compelling (Saikkonen et al. 2006, 2010a). On the other hand, the competitive superiority of endophyte-infected cultivars may cause negative side-effects when grown in mixtures with desirable pasture species e.g. legumes. A decrease in the abundance of legumes when grown in mixtures with endophyte-infected grasses compared with uninfected conspecifics has been reported in several studies (Hoveland et al. 1999; Malinowski et al. 1999; Takai et al. 2010). However, these endophyte-mediated impacts are context-dependent varying in environmental conditions, grass species and cultivars and genetic combinations of the fungus and the host grass (Dirihan et al. 2014).

The competitive superiority of endophyte-infected cultivars in nutrient rich agroecosystems should be regarded as having the potential for biological control of weeds (Saikkonen et al. 2013b). The breeding and use of endophyte-infected cultivars that do not produce mycotoxins but increase plant growth, seed production, seed germination rate and stress tolerance can increase productivity and prevent the use of herbicides in forage production (Gundel et al. 2013). Furthermore, possible allelopathic effects of endophyte-infected grasses on weeds could be used to prevent weed invasion. In set-aside fields, the use of endophyte-infected cultivars with high competitive ability has been demonstrated to slow weed invasion. Thus, in the long term, endophyte-infected grasses could be used to reduce the application of synthetic herbicides in grass and pastoral ecosystems and neighboring agricultural fields.

6.5.5 Summary of Endophyte Effects on Host Competitive Ability and Persistence

There is a continuum of interactions between grass and fungal endophytes from mutualism to antagonism depending on the grass species, plant-fungal genetic combination, and abiotic and biotic environmental factors. In mutualistic relations, endophyte-derived chemical compounds secreted into the soil by endophyte infected grasses may directly inhibit the growth of other species and neighboring plants. Higher survival, growth and reproduction of infected plants may also increase the superior productivity of infected grasses in ecosystems and maintain a high frequency of endophyte-infected grasses. This in turn may influence the composition of plant communities in natural- and agro-ecosystems. Although our knowledge on allelopathic effects of endophyte-infected grasses on weeds and volunteer species is low, it seems that there is the potential to use infected grasses for biological control of weeds in agroecosystems with minimum or no herbicide application.

6.6 Fungal Endophytes Support Host Plants in Overcoming Abiotic Ecological Constraints

Fungal endophytes of grasses are known to induce resistance in host plants to a range of abiotic stresses including drought, high soil salinity, heat, cold, oxidative stress, heavy metal toxicity and nutrient deficiency. It is this enormous diversity of effects, coupled with its vertical transmission, which makes fungal endophytes of grasses a stimulating field of study in agronomy and ecology. Several reviews and books dedicated to fungal endophytes of grasses and recording in detail their effects against abiotic stresses, have been published during the last 20 years (see Schardl 1996; Malinowski and Belesky 2000; Malinowski et al. 2005a; Cheplick and Faeth 2009). In this section, we feature recent progress in research (mainly between 2004 and 2015), and address its implications for the utilization of fungal endophytes of grasses in the ecological intensification of agriculture.

6.6.1 *Water Availability and Drought Stress*

Water scarcity due to drought and salinity stress affects more than 10 % of the total arable land areas of the world (Bartels and Sunkar 2005), and unsurprisingly it is the most documented abiotic stress in the grass-endophyte literature. Endophyte effects have been mainly investigated on the ability of two agronomically important grass species (perennial ryegrass and tall fescue) to grow and produce satisfactory yields under periodic drought (Saikkonen et al. 2006). An extensive body of research was produced on these symbioses for the purposes of characterizing the beneficial outcomes of endophytes on plants that could be used in grass improvement. Two decades of research show that endophyte effects on plants are more versatile than initially thought (Cheplick 2004; Müller and Krauss 2005; Saikkonen et al. 2006). The recent research on endophyte effects on native grasses also revealed that the effects of endophyte on host performance under water-limited conditions varied from one plant species and/or genotype to another (Morse et al. 2002; Ahlholm et al. 2002; Kannadan and Rudgers 2008; Rudgers and Swafford 2009). In fact, the endophyte effects appear to be dependent on environmental conditions and host-endophyte genotypic combinations.

In a recent meta-analysis, Chamberlain et al. (2014) highlighted the fact that biotic interactions are very often context-dependent, and suggested that the focus should be moved from ‘mean outcomes’ to the factors contributing to ‘variation in outcomes’. It remains unclear what factors contribute to the context-dependency in grass-endophyte symbioses (see Cheplick and Faeth 2009; Davitt et al. 2011), but field observations suggest a role for water availability in some species. Several surveys of native grasses have documented a higher frequency of symbiosis in drier

habitats in *Lolium perenne* (Lewis et al. 1997; Gibert et al. 2012), *Bromus setifolius* (Novas et al. 2007), *Festuca rubra* (Saona et al. 2010), the alpine grass *Festuca eskia* (Gonzalo-Turpin et al. 2010), and in many other *Lolium* and *Festuca* species in Europe (Leyronas and Raynal 2001). Therefore, water shortage in such grassland ecosystems may lead to higher infection rates among populations and a greater connection between growth and production in ecosystems to endophyte infection. In contrast, some other surveys show no correlation between infection rate and water availability in *Poa spiciformis* and *Phleum alpinum* in south Patagonia (Novas et al. 2007) or in native grasses from the California province (Afkhami 2012). These contrasting patterns suggest that the responsiveness of the endophyte-grass symbiosis may not depend only on environmental factors.

To expand our understanding of the variable effects of endophytes on their hosts, several authors have called for a better awareness of the origin of symbiotic plants in controlled experiments (Hesse et al. 2003; Kane 2011). Indeed, in grassland species (regardless of endophyte presence), adaptation of plants to drought has been consistently shown to depend on the origin of the genotypes (Pecetti et al. 2011; Annicchiarico et al. 2011), suggesting that drought resistance is associated with intra-specific variability. Experimental evidence of a positive effect of endophyte under drought have been highlighted by studies focusing on plants originating from dry environments and populations with different endophyte frequencies (Hesse et al. 2003; Gibert and Hazard 2011; Kane 2011; Gibert et al. 2012). For instance, Kane (2011) assessed the effects of the endophyte *Epichloë festucae* var. *lolii* on growth under stress of native *Lolium perenne* accessions originally collected from Italy, Morocco, Tunisia, and Turkey, areas where drought is known to occur. In four accessions, out of the six studied, endophyte-infected plants had more tillers, greater tiller lengths, total dry mass and green shoot mass under drought than endophyte-free plants, suggesting a positive effect of endophyte infection on host growth.

The occurrence of a high-infected population at a dry site is not evidence *per se* that the main advantage of endophyte occurs under drought. Indeed, Rudgers and Swafford (2009) tested the hypothesis that fungal endophyte *Epichloë elymi*, in the native grass species *Elymus virginicus*, would promote host growth under drought. In a growth chamber experiment, they imposed a 67 % reduction in water, corresponding to the very high end of drought predictions for the Midwestern US, where *E. virginicus* is common. Contrary to their initial prediction, they found that the positive effects of endophyte were stronger under the daily watering treatment than under drought: non-infected plants had 45 % less aboveground biomass than endophyte-infected plants under daily watering, and only 23 % less under drought. Similarly, Vázquez de Aldana et al. (2013a) showed no significant differences in response to the water treatment between endophyte-infected and endophyte-free plants in two populations of *Festuca rubra* from grasslands of western Spain originating from two contrasting habitat in terms of drought severity. Their results showed that *Epichloë festucae* did not increase the resistance of *Festuca rubra* plants to drought; there was no effect neither on plant biomass nor on proline content (a solute involved in osmotic adjustment, a strategy of drought tolerance). Instead,

differences in plant nutrition in response to water availability occurred, with endophyte-infected plants showing a significant increase in N, P and Zn in their shoot tissues, and Zn in roots relative to endophyte-free plants.

It seems that the intensity of drought experienced by the plant is an important parameter to consider when responses of perennial herbaceous species to drought are analyzed (Volaire et al. 2014). Different plant species are highly different with respect to their optimum environmental conditions, and a harsh event which is harmful for one plant species, might not be stressful for another. However, symbiotic plant performances under drought is usually characterized by drought resistance, *i.e.* the ability of plants to maintain a certain level of living biomass and growth under water shortage (Volaire and Lelièvre 2001). Drought resistance includes both drought stress avoidance and drought tolerance mechanisms, which both may be affected by fungal endophytes of grasses (for review see West 1994; Malinowski and Belesky 2000; Malinowski et al. 2005a; or more recently Swarthout et al. 2009). This criterion is relevant for most species of agronomic interest subjected to short and moderate periods of drought. However, when plants experience successive and severe summer droughts, their persistence is mainly determined by survival *i.e.* the ability of plants to remain alive during summer and recover when rehydration occurs (Volaire et al. 2001). It is now known that plant persistence during severe drought is governed by mechanisms different from those conferring resistance to moderate drought (Milbau et al. 2005).

Although drought survival in the grass-endophyte symbiosis has been much less explored than drought resistance, the few studies focusing on survival or recovery after stress support the view that endophyte benefits are greater during recovery from drought or during survival than during moderate water deficit (Malinowski and Belesky 2000; Hesse et al. 2003; Gibert and Hazard 2011). For instance, Gibert et al. (2012) estimated growth during drought stress and survival after severe stress for five populations of *Lolium perenne* along a gradient of water availability. Plant survival rates were highest in driest populations reinforced by fungal endophyte. Plants with a higher survival had lower growth, suggesting a trade-off between growth during stress and survival after stress. Consequently, the characterization of symbiotic plant stress responses should consider the intensity of stress experienced by the plants in the wild, and focus on the plant strategy potentially involved (resistance versus survival).

More and more studies have focused on the role of hybridization on the grass-endophyte symbiosis. Hybrid endophytes presumably result from somatic fusion of distinct *Epichloë* species infecting the same host individual (Scharndl and Craven 2003). The common idea is that hybrids may express traits from both ancestral species (Clay and Scharndl 2002), and thus may have an advantage in a larger range of environments than non-hybrid endophytes (Scharndl and Craven 2003). Several studies highlighted the abundance of hybrid endophytes among host species and populations (e.g. Iannone et al. 2009; Oberhofer and Leuchtmann 2012; Iannone et al. 2012), but the ecological consequences of endophyte hybridization have not been widely explored. In the native grass *Festuca arizonica*, hybrid endophytes are prevalent in habitats with low nutrients and moisture, whereas non-hybrid endophytes

are more prevalent in sites with higher nutrients and moisture (Sullivan and Faeth 2007; Hamilton et al. 2009). Under controlled experiments, hybrid endophytes of *F. arizonica* produced higher volume/mass ratio (Sullivan and Faeth 2007), higher biomass (Saari and Faeth 2012) and better competition and survival (Hamilton et al. 2010) than those having non-hybrid endophytes in stressful habitats (nutrients and water were manipulated). However, *F. arizonica* appears to be an exception among cool-season grasses by hosting mainly both hybrid and non-hybrid endophytes. In *Hordelymus europaeus*, a European woodland grass hosting preferentially hybrid endophyte, Oberhofer et al. (2014) found that non-hybrid endophytes prevailed at dry sites. In a controlled experiment, the authors showed no effect of hybridization on drought tolerance and plant survival and both hybrid and non-hybrid endophytes increased biomass and tiller production after recovery from stress. Yet, in this experiment, hybrid endophytes reduced or completely prevented plant reproduction compared with non-hybrid endophytes.

The last 5 years have been spent seeking evidence of water availability as a main cause of variability in the outcomes of grass-endophyte symbioses. This goal has been partially reached and water availability appears to be a driver of endophyte-grass symbiosis outcomes in some species, but the pattern of functioning appears undeniably more complex for other species. Beyond this unsatisfying conclusion, an interesting result is the effect of endophyte on both plant strategies of resistance and survival which is observed sometimes for the same host species (e.g. for *Lolium perenne*, Gibert et al. 2012, and Kane 2011). Since grasslands are expected to have production over several years, their sustainability is associated with both productivity and long-term persistence (Voltaire et al. 2014). Consequently, short-term adaptation in natural grasslands should include changes in species with greater resistance under periodic stress and/or greater survival under extreme events, two processes that may be improved by fungal endophytes of grasses (Fig. 6.3). The challenge is now to identify the most advantageous grass/endophyte combinations for local conditions.

6.6.2 Light Availability

In contrast with our current knowledge on drought, little is known about the effect of endophyte on grasses in response to shade. Yet, the response of plants to light availability is a key element for plant growth in mixed vegetation of tall and short plant species. Previous studies have recorded positive effects of endophytes on host plant growth under shade (Lewis 2004), and a higher concentration of alkaloids and phenolics under shade than under open sites in infected plants (Belesky et al. 2009). In a recent meta-analysis, Davitt et al. (2010) reported that approximately 25 % of symbiotic grasses were restricted to shady habitats versus only 12 % of non-symbiotic grasses. They also performed greenhouse experiments to study the effects of shade on the growth and traits of six perennial grass species (*Elymus villosus*, *Poa alsodes* and *Festuca subverticillata* occurring in shade, and tall fescue, *Poa*

(2013) showed that the beneficial effect of endophyte infection was less dependent on fertilizer supply than on water availability in *Leymus chinensis*, a dominant species native to the Inner Mongolia steppe. However, since nitrogen-availability appears to be a driver of alkaloid production in endophyte-infected grasses (Bylin et al. 2014), nitrogen-stress might be accompanied by herbivory stress in grasses, and should be studied in this context. On the other hand, in some dry land and over-grazed grass ecosystems, drought stress is accompanied by high concentrations of nitrogen; however the ways in which these conditions interact with *Epichloë* endophyte to alter grass growth and survival remains to be studied.

Whereas it is difficult to draw a clear and definite conclusion on endophyte functioning under metal stresses, studies have highlighted a large range of action of fungal endophytes on grasses (Table 6.4). For instance, fungal endophytes have been recently shown to increase tolerance to cadmium stress in *Achnatherum inebrians* (Zhang et al. 2010), salt-stress in *Hordeum brevisubulatum* (Wang et al. 2009) and in tall fescue (Sabzalian and Mirlohi 2010; Yin et al. 2014), Ni stress in tall fescue (Mirzahosseini et al. 2014) and arsenic stress in seedlings of *Festuca rubra* (Vázquez de Aldana et al. 2013b) (see Table 6.4). Unsurprisingly, the effect of endophyte on host plants is variable and has been shown to depend on the

Table 6.4 Effects of *Epichloë* fungal endophytes on host plants under some mineral/metal stresses

Mineral/ Metal stress	Grass	Endophyte species	Response of endophyte-infected grass under stress	Reference
Aluminum	<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	No effect on root and shoot dry matter More Al (47 %) and P (49 %) desorbed from root surfaces More Al (35 %) and P (10 %) concentrations in roots No differences in mineral concentrations in shoots	Malinowski and Belesky(1999)
	<i>Festuca rubra fallax, Festuca rubra rubra, Poa ampla</i>	<i>Epichloë festucae, Epichloë sp.</i>	Variable effect on root and shoot dry weight (positive to negative)	Zaurov et al. (2001)
	<i>Festuca arubra</i>	<i>Epichloë festucae</i>		
Arsenic	<i>Festuca arubra</i>	<i>Epichloë festucae</i>	No effect on germination response Longer radicles	Vazquez de Aldana et al. (2013c)

(continued)

Table 6.4 (continued)

Mineral/ Metal stress	Grass	Endophyte species	Response of endophyte-infected grass under stress	Reference
Cadmium	<i>Schedonorus arundinaceus</i>	<i>Epichloë sp.</i>	Increased tiller number and biomass under both control and Cd-stress conditions Increased Cd accumulation in host Improved Cd transport from the root to the shoot Higher phytoextraction efficiency (2.41-fold higher)	Ren et al. (2006)
	<i>Schedonorus arundinaceus</i> , <i>Schedonorus pratensis</i>	<i>Epichloë sp.</i>	Higher biomass production (12–24 %) Higher potential to accumulate Cd in roots (6–16 %) and shoots (6–20 %)	Soleimani et al. (2010)
	<i>Achnatherum sinebrians</i>	<i>Epichloë gansuensis</i>	More biomass and higher values for plant height and tiller number Increased antioxidative enzyme activities, H ₂ O ₂ concentration, and chlorophylls “a” and “b” levels Decline in proline and malondialdehyde content	Zhang et al. (2010)
	<i>Elymus dahuricus</i>	<i>Epichloë sp.</i>	Higher germination rate and index, and higher values for shoot length, root length and dry biomass	Zhang et al. (2012 b)
Copper	<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	Higher Cu concentrations in plants under greenhouse and field plot and pasture experiments	Dennis et al. (1998)

(continued)

Table 6.4 (continued)

Mineral/ Metal stress	Grass	Endophyte species	Response of endophyte-infected grass under stress	Reference
Nickel	<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	Higher plant growth in one genotype Improve in the antioxidative system in one genotype Reduction of Ni accumulation in the shoots of one genotype	Mirzahosseini et al. (2014)
Salt	<i>Hordeum brevisubulatum</i>	<i>Epichloë sp.</i>	Higher number of tiller, biomass accumulation Higher soluble sugar and proline contents, and superoxide dismutase isozyme activity Lower malondialdehyde content	Wang et al. (2009)
	<i>Schedonorus pratensis</i>	<i>Epichloë uncinata</i>	Higher leaf survival rates, root dry matter Reduced Na ⁺ and Cl ⁻ concentrations in roots Increased K ⁺ concentrations in the shoots	Sabzalian and Mirlohi (2010)
	<i>Achnatherum inebrians</i>	<i>Epichloë gansuensis</i>	Higher alkaloid levels	Zhang et al. (2011)
	<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	Higher Na accumulation and improved Na transport from the roots to the shoots Higher phytoextraction efficiency (2.34-fold higher) Higher tiller number, shoot height and total biomass	Yin et al. (2014)

(continued)

Table 6.4 (continued)

Mineral/ Metal stress	Grass	Endophyte species	Response of endophyte-infected grass under stress	Reference
Zinc	<i>Lolium perenne</i>	<i>Epichloë festucae var lolii</i>	Decrease of growth less pronounced Lower Zn concentration (24–32 %) in leaves Greater quantum yield of electron flow through the photosystem II (PSII) Normal level of internal CO ₂ concentration Higher total dry weight, and tiller number	Monnet et al. (2001)
	<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	Higher chlorophyll fluorescence at high concentrations of Zn Greater concentration of Zn in shoots	Zamani et al. (2015)

(continued)

genotype of both partners (Vázquez de Aldana et al. 2013b; Mirzahosseini et al. 2014), or the level of stress experienced (Zhang et al. 2010). Despite this variability in the symbiosis outcomes, it seems that fungal endophyte will open up new alternative strategies for phytoremediation of heavy metals and desalinization processes in the future of agroecosystem management.

6.6.4 Summary of Endophyte Support under Abiotic Ecological Constraints

Fungal endophytes support host plants and allow them to overcome abiotic stresses including drought, light, mineral and metal stresses. Recent evidence about the effect of fungal endophyte on drought survival, suggest that this symbiosis would be used for both productivity and stability and long-term persistence of grasslands. In addition, evidence of the role of fungal endophytes on plant resistance to metal/mineral stress suggest their large potential for phytoremediation of heavy metals and desalinization processes. However, the context dependency of the

grass-endophyte symbiosis is still problematic for a general use of these symbioses across different plant species or environmental conditions. The challenge is now to identify the combination of grass-endophyte that will be the most advantageous for local conditions (environmental and cultural conditions).

6.7 Endophyte Modifies Biomass and Energy in a Grassland Ecosystem

The ability of *Epichloë* endophytes to impart beneficial properties to the host grass, resulting in improved plant growth and persistence, has been most intensely utilized in the mesic and managed grassland ecosystems of USA, New Zealand and Australia. In these regions, tall fescue and/or perennial ryegrass introduced from Europe have become naturalized in large areas and are the preferred species in many sown pastures as they display a wide range of adaption to climates, soils and management (Jung et al. 1996; Fribourg et al. 2009). Depending on the grass species and region, endophyte infection can be ubiquitous in naturalized populations and is preferred in sown seed due to the agronomic benefits. Unlike some other aspects of *Epichloë* endophyte infection which have been studied in controlled conditions, the effect on plant biomass and the subsequent energy input into the ecosystem has been extensively studied in field experiments.

6.7.1 USA

6.7.1.1 Continental-Type Tall Fescue

Continental-type tall fescue (Hand et al. 2010) is the most widely used temperate grass species in the USA, occupying some 14 million ha of pasture lands in parts of the Pacific northwest but mostly in the eastern USA (Young et al. 2013). While its range of adaptation is large, its predominate use is in the transition zone of the upper South and lower Midwest, an area commonly termed the ‘fescue belt’ reflecting the dominance of this grass in pastures, roadsides and amenity areas. Within the fescue belt, it is generally accepted that endophyte is necessary for tall fescue persistence in the southern half of this area. Abiotic stresses occur widely in this region, as droughts and high temperatures occur in summer on soils of low water holding capacity and poor nutrient status as the soils are highly weathered, eroded and shallow (Belesky and West 2009). Through a variety of morphological, biochemical and physiological mechanisms, endophyte in tall fescue has been shown to confer tolerance to both soil water deficit and some nutrient stresses (see Sect. 6.6). In addition, endophyte-enhanced growth in this region is likely due to protection from biotic stresses such as insects and nematodes (Popay 2009a; Timper 2009 and for more information see Sect. 6.3) and reduction in overgrazing in the case of common toxic

endophyte (Waller 2009). Even in unmanaged grassland ecosystems that are free of grazing livestock, endophyte has been shown to have a beneficial effect on persistence of infected grasses (Clay et al. 2012).

Increases in persistence and productivity due to endophyte infection of tall fescue have been demonstrated widely across the south eastern states of the US (Belesky and West 2009). In northeast Texas, Read and Camp (1986) measured 35 % less available forage on low endophyte (8 % infection) than highly endophyte-infected (95 % infection) paddocks of tall fescue over 2 years of cattle grazing, and failure of two out of three low endophyte paddocks to survive the final summer. In Georgia in a 3-year cutting experiment with four tall fescue populations, Bouton et al. (1993) reported a range of endophyte effects depending in location. In the most extreme case at Americus, endophyte-infected populations (greater than or equal to 85 % infection) in year 1 produced twice the herbage yield of endophyte-free and by autumn, endophyte-free plots had failed to persist (Table 6.5). Endophyte effects were more moderate at Tifton, where agronomic advantages to endophyte infection became more consistent with increasing time, and by autumn of year 3 endophyte-infected plots had twice the yield and ground cover of endophyte-free plots. At Watkinsville, no differences in yield and persistence were observed. These differences between locations in Georgia corresponded with the severity of summer drought, being greatest at Americus. Summer soil water deficit was also important in determining the advantage to endophyte infection in northwest Arkansas (West et al. 1993). At this location, in a dry year under no irrigation, tiller numbers in endophyte-free plots only recovered to 62 % of the numbers in endophyte-infected (80 % infected) plots, and these differences continued into the following year. While herbage yields did not consistently exhibit the same endophyte differences, possibly due to greater tiller size in endophyte-free plants, the weed contents in non-irrigated plots free of endophyte were twice as high as endophyte-infected. Bouton et al. (2002) also reported selected non-toxic endophytes largely enhanced the productivity and persistence of tall fescue comparable with what is seen for the common toxic endophyte.

6.7.1.2 Mediterranean-Type Tall Fescue

Use of tall fescue of Mediterranean origin and growth pattern (Hand et al. 2010) has the potential to extend the range of tall fescue into southern California and the sub-humid to semiarid transition zone of the southern Great Plains of Oklahoma and Texas (Young et al. 2013). Through summer dormancy, this type of tall fescue can survive hot, arid summers where Continental-type tall fescue fails (Malinowski et al. 2005b). Given the positive effects that endophyte has in enhancing the agronomic performance of Continental-type tall fescue, particularly in the southern USA, the role endophyte may have in expression of this summer dormancy, and the overall productivity of the host grass, is of interest.

Mediterranean-type tall fescues collected from countries of the Mediterranean basin are commonly highly infected with endophyte. In three studies of 211 seed

accessions collected from Morocco, Tunisia, Sardinia and Greece, endophyte was present in 89 % of accessions, with mean plant infection rates of greater than 57 % (Clement et al. 2001; Piano et al. 2005; Takach et al. 2012). High infection rates are a strong indication that under the abiotic and biotic stresses of these environments, endophyte infection enhances persistence of the host tall fescue plant. In addition, bioactivity has been demonstrated by Clement et al. (2001) with resistance to the bird cherry-oat aphid (*Rhopalosiphum padi*) occurring in most endophyte-infected accessions. However, mammalian toxic alkaloids may be produced by many of these associations (Takach et al. 2012; Piano et al. 2005).

Field testing of Mediterranean-type tall fescue at Vernon, Texas has shown variable agronomic effects of endophyte depending on year and plant population. At this site, Malinowski et al. (2005b) reported no effect of endophyte on dry matter yield of cv. Grasslands Flecha over 3 years, but a 22 % increase due to endophyte in a year following a year of extreme drought, and 21–50 % greater numbers of tillers surviving drought in 2 years. Furthermore, Malinowski et al. (2012) found endophyte responses for the TX06V population for plant persistence, herbage yield, dormancy rating, and competitive ability, but this was not the case for Flecha. In contrast, at this site Thomas et al. (2013) found no effect of endophyte presence in summer on the survival, shoot dry matter yield, leaf senescence, tiller-base water content, or a range of biochemical parameters for TX06V-B-FA and Flecha tall fescues.

Despite these variable field results in Texas, indoor studies have shown endophyte presence can improve mineral nutrition in both Mediterranean and Continental-type tall fescues (Malinowski et al. 2012) which may result in better agronomic performance in situations of limited soil nutrients. In addition, extensive testing in Australia has shown infection with the selected, non-toxic ‘AR542’ endophyte improves agronomic performance relative to endophyte-free in a majority of experiments for a range of cultivars of both Mediterranean- and Continental-type tall fescues (Hume and Sewell 2014). There is a need for a greater understanding of the environmental drivers for endophyte-enhanced growth and persistence of tall fescue and how these differ between regions/countries and years.

Table 6.5 Effects of common toxic endophyte on yield and persistence in the first year of production (1988) of tall fescue (*Schedonorus arundinaceus*) in the southern zone of the ‘fescue belt’ at Americus, Georgia, USA

Infection status of tall fescue	Yield (kg DM/ha)				Stand persistence (% ground cover)	
	Winter	Spring	Autumn	Total	Late spring	Late autumn
Common toxic endophyte	378 a	5792 a	3510 a	9680 a	75 a	91 a
No endophyte	260 b	4601 b	0 b	4861 b	61 b	0 b

Plots were sown in October 1987. Data presented is pooled data for four populations. Within columns, means followed by the same letter are not significantly different ($P < 0.05$). Adapted from Bouton et al. (1993)

6.7.1.3 Ryegrass

In contrast to tall fescue, use of perennial ryegrass in pastures is limited to only 110,000 ha, being grown in the cooler, moister or irrigated northern latitudes of the USA (Young et al. 2013). Cultivars for use in forage are mostly endophyte-free, with no claims being made by US seed companies about endophyte levels or strain. There appears to be an absence of published information on agronomic field experiments to test if endophyte enhances persistence and production in these environments. However, within the amenity turf industry, it is widely accepted by breeders and practitioners that endophyte enhances persistence and performance of perennial ryegrass (and tall fescue) largely through enhanced insect tolerance (Brilman 2005; Young III and Silberstein 2012). Although level of endophyte in seed is not regulated, in 2006 over 73 % of turf perennial ryegrass cultivars in the USA had high (greater than 60 % infected seeds) infection levels (Young III and Silberstein 2012). It would therefore seem reasonable that endophyte could enhance the agronomic performance of ryegrass in pastures in the USA, dependent on the severity, frequency and type of biotic and abiotic stresses. If this is the case, selected endophytes should be used with low or no toxicity to livestock (see Sect. 6.2) (Young et al. 2013; Johnson et al. 2013).

6.7.2 *Australia and New Zealand*

6.7.2.1 Tall Fescue

In Australia and New Zealand, tall fescue utilized in sown pastures has been largely free of endophyte (Easton et al. 1994). It has long been known that endophyte-infected plants that occur on occasions in pastures, waste lands and alongside water ways can cause toxicity to livestock due to infection with common toxic endophyte (see Sect. 6.2). Endophytes to enhance agronomic performance have been therefore selected with no toxicity to sheep and cattle, and these are currently available to farmers (Young et al. 2013; Hume and Sewell 2014).

In New Zealand, the role endophyte may have in improving agronomic performance of tall fescue was first seen in Northland in soils that are free draining, suffer from summer drought and harbor damaging populations of African black beetle (Hume and Barker 2005) (Fig. 6.4). In a small plot agronomic experiment, endophyte infection increased from 30 % at sowing to 80 % of tillers infected after 4 years. Further small plot testing over 10 years in Northland with a range of Continental- and Mediterranean-type tall fescue cultivars showed increases of up to 66 % in plant numbers and annual dry matter yields, with differences being greatest in late summer-autumn (Hume et al. 2009). Greater persistence of endophyte-infected tall fescue which is more competitive than lower quality C4 grasses, compared with endophyte-free, has been seen on farms in this region under cattle grazing. Similar levels of endophyte-enhanced growth of tall fescue have been



Fig. 6.4 Perennial ryegrass (*Lolium perenne*) and tall fescue (*Schedonorus arundinaceus*) small plots in autumn near Bega, New South Wales, Australia (4 years post sowing). Differences in persistence predominately due to damage from African black beetle (*Heteronychus arator*) (Photo courtesy of David Hume, AgResearch Ltd., New Zealand)

recorded in other regions of New Zealand, with the advantage to endophyte varying between regions corresponding closely to the severity of both soil water deficit and insect pest pressure particularly in the summer-autumn period (DE Hume unpublished data).

Agronomic evaluation of selected endophytes has occurred throughout the south eastern states of Australia where within the same cultivar, endophyte-free has been compared with the selected 'AR542' endophyte in more than 30 experiments since 2000 (Fig. 6.4) (Hume and Sewell 2014). Over 80 % of these experiments showed agronomic advantages to the AR542 endophyte, which were typically in the range of +8 % to +100 % (mean +38 %, median +30 %) for both Continental- and Mediterranean-types of tall fescue. In the most extreme case in the Hunter Valley of New South Wales, endophyte-free cultivars had completely failed to survive 1 year after sowing while those infected with AR542 were dense and productive. In many cases, the specific environmental drivers behind these differences were unclear, but in general, differences occurred and were largest when soil, insect, and climate/weather stresses were at their greatest, similar to what has been seen in New Zealand and the USA. When these biotic and abiotic stresses were lowest, tall fescue cultivar rather than endophyte was the major determinant of agronomic performance.

Despite the useful agronomic attributes of tall fescue, particularly under harsh soil and climatic conditions where perennial ryegrass struggles, its use in the past has been very limited in New Zealand and restricted in Australia (Easton et al. 1994). For example, tall fescue in Australia is grown on 1.1 million ha, only 7 % of its potential adaptive area. Given the agronomic evidence from comparisons of endophyte-infected and endophyte-free cultivars, it is highly likely that elite cultivars with non-toxic selected endophytes will broaden the useful range of tall fescue, increasing pasture and overall farm productivity in both Australia and New Zealand (Young et al. 2013). Models to predict where and when endophyte infection would be of agronomic and financial benefit to farmers may assist in uptake of selected endophyte technology. In the case where new pastures are being sown due to crop rotation or pasture renewal, selected endophyte technology can be deployed for only a small marginal increase to the overall cost of establishment.

6.7.2.2 Ryegrass

The situation in Australia and New Zealand for perennial ryegrass parallels that of the USA for tall fescue, as endophyte-infection is essentially the norm and research focused initially on the agronomic impact of removing the common toxic endophyte due to its clinical and subclinical toxicity to livestock (see Sect. 6.2). The agronomic importance of endophyte infection in New Zealand was first determined 1 year after the link had been established between endophyte and ryegrass staggers in sheep. In early 1982 in a field experiment at Hamilton in northern New Zealand, endophyte-infected plots produced threefold more dry matter in late summer-early autumn than endophyte-free plots (Mortimer et al. 1982). Endophyte-free ryegrass had been severely damaged by Argentine stem weevil, a major insect pest of ryegrass. Unlike the USA, this dispelled the concept of deploying endophyte-free seed as the solution to endophyte livestock toxicity in ryegrass, as the negative impact on yield and persistence of ryegrass and so ultimately the total productivity of the pasture was considered to be too great.

Further field experiments examining the impact of endophyte on agronomic performance continued to demonstrate the important role endophyte had in ensuring the high yield and persistence of ryegrass pastures in many but not all cases, in both New Zealand and Australia. For example, in a national series of 10 small plot experiments sown in four regions of New Zealand, endophyte infection enhanced yields in all experiments, predominately in the period of mid-summer to mid-autumn period, in some cases by up to 88 % (Popay et al. 1999). Under dairy grazing in Waikato, New Zealand, endophyte-free ryegrass pastures were so severely depleted that they needed to be resown to restore them to an agronomically productive level (Thom et al. 2014). In Australia, over half of 18 experiments had advantages of +7 % to +212 % (mean +44 %, median +29 %) to endophyte-infected plots (Fig. 6.4) (Hume and Sewell 2014). However, in areas or years of low insect damage and cool moist summers (and/or irrigation) there may be no agronomic advantage in the short term to endophyte infection.

Selected endophytes of no or low toxicity to livestock (see Sect. 6.2) have varying impacts on agronomic performance (Popay and Hume 2011). This has been most clearly demonstrated in New Zealand, but similar evidence is accumulating in Australia. The key driver behind these differences is the breath of protection each endophyte strain imparts to the ryegrass from damage by up to five of the six major exotic and native insect pests in New Zealand. Strain differences are most pronounced when damage from high pest populations occurs when ryegrass is under stress from heat and soil water deficits in summer/autumn (Popay et al. 1999; Popay and Hume 2011; Thom et al. 2014). Under these conditions, AR37-infected ryegrasses have the best agronomic performance in both New Zealand and Australia (Hume et al. 2009; Popay and Hume 2011; Hume and Sewell 2014).

Effects of endophyte infection on overall agronomic performance of the pasture are less well documented than the effects solely on the endophyte-infected host grass. This is primarily due to the sown grass component of a mixed sward often being the major driver of total yields and to a large extent quality of the pasture. In some cases, catastrophic collapse of endophyte-free ryegrass in mixed pastures necessitates the resowing of these fields, e.g. Thom et al. (2014). In other cases, endophyte infection of ryegrass may have relatively little impact on total pasture production, but undesirable changes occur in species composition due to ingress of broad-leaf weed species and grasses species of poor feed quality and/or agronomic performance. For example, in sub-tropical, south-east Queensland, Australia, irrigated-plots sown as pure ryegrass differed little in total dry matter yields over 3 years between endophyte-free (40.03 t DM/ha) and common toxic endophyte-infected ryegrass (41.02 t DM/ha) (Table 6.6) (Lowe et al. 2008). However, undesirable C_4 grasses were 55 % higher in endophyte-free than endophyte-infected plots, reducing the forage quality of the sward.

White and subterranean clovers (*Trifolium repens* and *T. subterraneum*, respectively) are important components of managed grasslands in Australia and New Zealand. While their overall contribution to total sward production is small relative to the sown grass, clovers produce highly nutritious feed for livestock and provide nitrogen to the pasture through symbiotic rhizobia that fix nitrogen from the atmo-

Table 6.6 Effects of common toxic endophyte on plant persistence and yields of irrigated perennial ryegrass (*Lolium perenne*) in subtropical, southeast Queensland, Australia

Infection status of ryegrass	Plant persistence (% frequency)				Yield (t DM/ha)	
	End of summer			End of autumn	Total 3-year ryegrass	Total 3-year weeds
	Year 1	Year 2	Year 3	Year 3		
Common toxic endophyte	61.2 a	49.0 a	16.7 a	15.6 a	33.69 a	7.33 b
No endophyte	48.1 b	40.1 a	5.6 b	5.0 b	28.67 b	11.36 a

Within columns, means followed by the same letter are not significantly different ($P < 0.05$). Adapted from Lowe et al. (2008)

sphere. In general, any factor that enhances ryegrass growth, such as nitrogen fertilizer and grazing management, results in ryegrass being more competitive for limited soil and light resources, so clover growth and content may decline in the short term. Endophyte-infected ryegrass, compared with endophyte-free ryegrass, has on occasions been shown to reduce clover contents in the field, most likely through greater competitive ability but also possibly through allelopathic effects that endophyte-infected grasses have over other plant species (see Sect. 6.5) (Sutherland et al. 1999; Thom et al. 2014). In some cases this can be substantial, with Cunningham et al. (1993) reporting subterranean clover contributing 30 % of the yield in endophyte-free ryegrass pastures but close to zero in endophyte-infected pastures in southwest Victoria, Australia. However at the same site over 3 years, Quigley (2000) reported no effect of endophyte infection in ryegrass on subterranean clover plant numbers despite 37 % and 7 % more ryegrass plant and tiller numbers and 10 % more total dry matter yields in endophyte-infected pastures.

6.7.3 Summary for Endophyte Effects on Biomass and Energy in a Grassland

There is compelling evidence that suggests *Epichloë* endophytes improve the medium to long term agronomic performance of tall fescue and perennial ryegrass pastures in large areas of the mesic, managed grasslands of the USA, New Zealand and Australia. The advantages of endophyte infection is greatest with edaphic stresses (e.g. drought) combined with biotic stresses (e.g. insect pests). These data support the widespread commercial use of endophyte-infected cultivars in these countries. While endophyte-infected cultivars have enhanced persistence and yield, and weed contents of swards are reduced, in comparison with equivalent endophyte-free cultivars, farmers need to be aware that the performance of companion clovers could be compromised on occasions.

There are intriguing differences between these countries as to whether tall fescue and ryegrass have naturalized as infected with animal-toxic endophyte strains, or are endophyte-free. In the case where endophytes are toxic to animals, cultivars with selected endophytes can be utilized. These selected endophyte-infected cultivars may have varying agronomic performance relative to the common toxic endophyte-infected cultivars, but they represent a significant economic gain for farmers through good agronomic performance with no or reduced adverse effects on grazing animals. Where in the past endophyte-free cultivars have been used, for example tall fescue in New Zealand and Australia and ryegrass in USA, use of selected endophytes provided an opportunity to enhance agronomic performance and also broaden the useful range of these valuable pasture grasses. The deployment of selected endophytes in Mediterranean-type tall fescue cultivars in Australia and USA provides an opportunity to use endophytes to boost the agronomic performance of tall fescue in regions with hot, arid summers. There is a need for a greater understanding of the environmental drivers for endophyte-enhanced growth and

persistence of Mediterranean tall fescue and how these differ between regions/countries and years. Opportunities exist beyond these countries to improve the performance of tall fescue and ryegrass through the use of selected endophytes in cultivars adapted to these regions.

6.8 Global Change Impacts on Grass-Endophyte Interactions

6.8.1 Global Change Variables

Atmospheric CO₂ is expected to increase to between 450 and 950 ppm by the year 2100 with subsequent radiative forcing expected to increase global mean surface temperature by 1–3.7 °C (IPCC 2013). Altered precipitation and temperature-induced reductions in soil water content are likely to enhance drought in some regions (IPCC 2013). Climate variables are expected to alter the physiology and phenology of plants, animals and fungi, with broader consequences for species interactions such as herbivory, competition and mutualism.

6.8.2 Global Change Impacts on the Ecology of Grass-Endophyte Interactions

The effects of climate variables such as CO₂ and warming have not been widely studied in *Epichloë* endophytes, though effects on other fungal mutualists of plants have been well documented. Studies have shown that global change variables can impact the ecology of plant-fungal symbiont interactions both by directly altering fungal growth (Compant et al. 2010) and through fungal symbiont-mediated changes in plant growth responses (Kivlin et al. 2013). In a review of plant mutualisms and climate change, Compant et al. (2010) found that both temperature and CO₂ enhanced the colonization of arbuscular and ectomycorrhizal fungi. For CO₂, this is likely due to enhanced carbon resources from higher photosynthetic output, and a similar observation might be expected in other carbon-limited fungal mutualists. Changes in plant nutrient allocation may be especially important for plant-fungal mutualisms where nutrient exchange forms the basis of species interactions.

Table 6.7 shows the literature to date on changes in endophyte and alkaloid concentrations in response to experimental warming and CO₂ increase. In most cases, experimental warming resulted in increased endophyte prevalence and concomitant increase in alkaloid concentration, and this is consistent with evidence from field studies. di Menna and Waller (1986) found that pastures of *Epichloë*-infected *Lolium perenne* had higher hyphal density in mid-summer when temperatures were highest. Similar seasonal fluctuations were noted for *Epichloë*-infected tall fescue

Table 6.7 Studies that have investigated the effects of elevated atmospheric CO₂ or temperature on grass-endophyte interactions in controlled environments

CO ₂ studies		% Concentration change							Reference
Plant species	<i>Epichloë</i> endophyte species	Treatment levels	Lolines	Ergots	Lolitrems B	Peramine	Endophyte ^a		
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	396 ppm +300 ppm	-25.7	-31.4			+12.3	Brosi et al. (2011)	
<i>Bromus erectus</i>	<i>Epichloë bromicola</i>	360 ppm					NS	Groppe et al. (1999)	
<i>Lolium perenne</i>	<i>Epichloë festucae</i> var. <i>lolii</i>	368 ppm		+819.5		+196.8		Hunt et al. (2005) ^b	
<i>Lolium perenne</i>	<i>Epichloë festucae</i> var. <i>lolii</i>	368 ppm		NS		NS		Hunt et al. (2005) ^c	
<i>Bromus erectus</i>	<i>Epichloë bromicola</i>	350 ppm					+53.1	Meijer and Leuchtman (2000) ^d	
<i>Bromus erectus</i>	<i>Epichloë bromicola</i>	350 ppm					-37.2	Meijer and Leuchtman (2000) ^e	
<i>Bromus erectus</i>	<i>Epichloë bromicola</i>	350 ppm					NS	Meijer and Leuchtman (2000) ^f	
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	390 ppm	-18.2	-13.4		NS	+36.6	Ryan et al. (2014a)	
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	390 ppm	NS	NS		NS	NS	Ryan et al. (2014a)	

(continued)

Table 6.7 (continued)

Temperature studies		% Concentration change							Reference
Plant species	<i>Epichloë</i> endophyte species	Treatment levels	Lolines	Ergots	Lolitrems B	Peramine	Endophyte ^a		
<i>Lolium perenne</i>	<i>Epichloë festucae</i> var. <i>lolii</i>	10 °C 20 °C					+53.2	Breen (1992)	
<i>Lolium perenne</i>	<i>Epichloë festucae</i> var. <i>lolii</i>	14 °C 21 °C					NS	Breen (1992)	
<i>Lolium perenne</i>	<i>Epichloë festucae</i> var. <i>lolii</i>	21 °C 28 °C					NS	Breen (1992)	
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	ambient +3 °C	+25.7	NS			NS	Brosi et al. (2011)	
<i>Lolium perenne</i>	<i>Epichloë festucae</i> var. <i>lolii</i>	20 °C		+105.3	NS	NS		Eerens et al. (1998)	
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	21/15 °C 32/27 °C	-63.5					Kennedy and Bush (1983)	
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	10 °C					+288.2	Ju et al. (2006)	
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	15 °C 20 °C					+306.1	Ju et al. (2006)	
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	12/6 °C 25/19 °C					+3578.9	Ju et al. (2006)	

<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	ambient	+3 °C	NS	NS	NS	McCulley et al. (2014) ^g
<i>Schedonorus arundinaceus</i>	<i>Epichloë coenophiala</i>	ambient	+3 °C	+30.9	+40.7	NS	McCulley et al. (2014) ^h
<i>Lolium perenne</i>	<i>Epichloë festucae</i> var. <i>loli</i>	10/10 °C	20/10 °C	NS	+3293.1	+128.8	Ryan et al. (2015)

Changes in alkaloid and endophyte concentrations were extracted from the listed references and are expressed here as % concentration change relative to controls. In cases where there were two-way interactions between CO₂/temperature and other variables, the main effects are reported the other variable (e.g. year). Otherwise, the main effects are reported

^aEndophyte concentration studies included those measuring infection frequency (Brosi et al. 2011; Ju et al. 2006; McCulley et al. 2014), number of endophytic cells using qPCR (Groppe et al. 1999; Ryan et al. 2014a; Ryan et al. 2015), hyphal counts (Breen 1992), and choke rates in parasitic *Epichloë* species (Meijer and Leuchtmann 2000).

^bMeasured at 40 mol/m³ nitrogen

^cMeasured at 10 mol/m³ nitrogen

^dNo fertilizer 1996

^eHigh fertilizer 1996

^f1997

^gMeasured in Spring

^hMeasured in Fall; NS = not significant; Blank cells = parameter not measured

where both infection frequency (Ju et al. 2006) and alkaloids (Kennedy and Bush 1983) increased in mid-summer.

Two studies found that *Epichloë*-derived alkaloids decreased in tall fescue grown in elevated CO₂ (Table 6.7). In contrast, Hunt et al. (2005) found increases in alkaloid concentrations in perennial ryegrass grown in high CO₂ and high nitrogen fertilization, though those grown in nitrogen poor conditions showed no change in alkaloids. As Table 6.7 suggests, the disparities within the CO₂ literature may be due to plant and fungal species, CO₂ treatment levels, and plant nitrogen status. Endophyte concentration tended to increase under elevated CO₂ (Table 6.7) though several studies have found no change and one study found decreased concentrations in a high fertilization treatment. The observation that endophyte growth and transmission may increase while alkaloid production decreases may have consequences for the strength of mutualistic interactions in the future.

6.8.3 *Impacts of Global Change on the Utility of Fungal Endophytes in Agroecosystems*

Epichloë-infected cool-season grasses have high agronomic importance due to increased resistance to stresses such as drought and insect herbivory. As such, novel associations (see Sects. 6.2 and 6.3) containing alkaloid profiles that reduce toxicity to grazing vertebrate herbivores, but maintain invertebrate toxicity, have been widely marketed for pest control in forage crops. Insect herbivore performance is generally reduced in high CO₂ due to lower plant concentrations of limiting nutrients such as nitrogen, though responses have been shown to depend on insect feeding guild (Robinson et al. 2012). Few studies have examined grass-endophyte-insect interactions in the context of climate change. Ryan et al. (2014a, b) found that while alkaloid concentration was reduced in high CO₂, aphid colonization on endophyte-infected tall fescue was consistently low, regardless of CO₂ concentration. Similarly, Marks and Lincoln (1996) found that while the growth rate of fall armyworm was reduced by endophyte infection, there was no CO₂ × endophyte interaction. This may be due to simultaneous endophyte-mediated changes in both primary and secondary plant metabolism, which can alter host quality for insects in complex ways. Hunt et al. (2005) found that a CO₂-induced decrease in soluble proteins in uninfected plants was not observed in infected conspecifics, suggesting that endophyte infection may mediate changes in plant primary metabolism in ways that can affect insect diet quality.

Insect pests are likely to respond to warming through both direct (i.e. physiological) and indirect (i.e. plant-mediated) mechanisms and indirect changes in host plant quality can be mediated by endophytes. Salminen et al. (2005) found that fall armyworm performance was lower when fed endophyte-infected grass tissue that had been grown in higher temperatures relative to material that had been grown in lower temperatures, suggesting that warming may provide enhanced resistance in

endophyte-infected hosts. However, direct effects of warming are likely to increase the number of generations of multivoltine insects in a given year due to increased development rate and longer growing season (Bale et al. 2002). Thus, increased insect herbivore loads in some regions may increase the competitive advantage of endophyte-infected grasses, particularly in light of preliminary observations that warming may increase endophyte and alkaloid concentrations (Table 6.7).

Endophyte-infection and climate variables can interact in ways that affect forage crop yields. Marks and Clay (1990) found that biomass increased by 80 % under elevated CO₂ in perennial ryegrass infected with *Epichloë festucae* var. *lolii* compared with only a 21 % increase in uninfected plants. However, no CO₂ × endophyte interaction was found for yield responses in tall fescue (Chen et al. 2007; Brosi et al. 2011; Ryan et al. 2014b). The advantages of endophyte infection for drought avoidance, tolerance, and recovery in host plants have been extensively documented (Malinowski and Belesky 2000). A recent meta-analysis by Kivlin et al. (2013) found that fungal symbiont associations in general (leaf endophytes, arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and dark septate endophytes) were able to ameliorate the negative effects of drought on host plants associated with climate change. In future climates, such adaptations could be beneficial for plant growth and persistence in soils where water is a growth-limiting factor. In addition, some alkaloids have previously been shown to increase under drought stress (Kennedy and Bush 1983; Belesky et al. 1989; Brosi et al. 2011), which may result in increased protection against insect herbivory in future climates when insect outbreaks are hypothesized to increase (Bale et al. 2002).

6.8.4 Risks, Opportunities, and Future Directions

Table 6.7 highlights the paucity of work that has been done in the area of grass-endophyte responses to climate change. Most studies on endophyte responses to temperature were not designed to directly address hypotheses related to climate change though information about potential responses might be inferred from such work. Only two studies (Brosi et al. 2011; McCulley et al. 2014) have examined endophyte response to warming treatments in the range of projected global mean temperature increases (in both cases ambient +3 °C). These studies found that while endophyte infection frequency was similar between ambient and elevated temperatures, alkaloids tended to increase in response to warming, though such increases were dependent on alkaloid group (Brosi et al. 2011), season (McCulley et al. 2014), and precipitation level (McCulley et al. 2013). Thus while some general responses are beginning to emerge, it is not yet clear how these suite of changing variables may interact. There is also evidence that climate change effects on grass-endophyte interactions may also interact with variables such as UVB (Newsham et al. 1998) and mineral fertilization (Hunt et al. 2005; Kivlin et al. 2013).

More research is needed to identify potential risks and opportunities associated with grass-endophyte interactions in a changing climate and to inform future

management practices and breeding programs. Increased alkaloid toxicity in warmer climates may benefit pest management in forage crops. However, alkaloid toxicity has been associated with poor animal performance during summer grazing when alkaloid concentrations tend to increase (Kennedy and Bush 1983) and this phenomenon is likely to be exacerbated by climate change. For example, recent outbreaks of severe ryegrass toxicosis in Australia have in part been attributed to warmer springtime temperatures (Reed et al. 2011). As such, breeding for low toxicity strains and/or novel associations will likely form a critical part of adaptive responses. Selection of grass-endophyte combinations that maximize drought tolerance and recovery will likely benefit regions of higher drought incidence in future climates. It has been suggested that breeding and bioengineering programs could be undertaken to take advantage of high CO₂ (Ainsworth et al. 2008) and such strategies could also be used for pasture productivity. In addition, research has shown that high endophyte pastures are able to sequester more carbon than endophyte-free pastures, suggesting that endophytes may even have a role in climate mitigation (Iqbal et al. 2012).

6.8.5 Summary of Grass-Endophyte Responses to Global Change

Changes in CO₂ concentration, water availability, and temperature are likely to affect grass-fungal mutualism interactions in the future. While the effects of drought responses on grass-endophyte interactions have been widely studied, much less is known about how these species will respond to rising CO₂ and temperature. Research to date suggests that endophyte growth (tiller infection frequency and endophyte concentration) and alkaloid concentrations may increase in response to temperature, and this is consistent with field studies showing increased endophyte-associated toxicity in summer months. Alkaloid and endophyte responses to CO₂ have been less consistent, and preliminary research suggests that responses are dependent on plant and fungal species, and plant nitrogen status. The effects on agroecosystems are likely to be complex. Some research suggests that endophyte-infected grasses have higher yield responses to CO₂ than endophyte-free grasses, though this effect appears to be species-specific. Additionally, endophyte infection may buffer yield losses associated with drought in some regions. However, such benefits will need to be weighed against the possibility of increased toxicity for grazing animals, a phenomenon that is already occurring in some parts of Australia.

6.9 Conclusion

Most of the information available on ecological consequences of *Epichloë* fungal endophytes pertains to two important grass species, tall fescue and ryegrass, and mainly from a few pioneer countries in endophyte research. Lower live weight gains

and neurological symptoms in grazing animals is still a common problem in plains predominantly covered by these two species infected with common toxic *Epichloë* endophytes. However, changes in management practices to utilize selected strains of *Epichloë* and searching for new isolates with no or minimum animal toxicity, not only let farmers get rid of potential animal disorders, but also help pastures and grasslands become more productive and stable over time.

In contrast to natural conditions, in agroecosystems a particular grass-endophyte combination could be propagated and maintained to increase productivity and stress resistance and to shape the growing area. This has been done to decrease the negative impact of *Epichloë* toxic alkaloids on grazing animals in sown pastures. For some environments like saline soils or environments under heavy metal stress, some other strains of endophyte may be more appropriate to be used as symbionts of grasses. Screening a wide variety of grass-endophyte genotypes is a prerequisite to exploring the combinations in pre-determined environments which could be used for specific applications in agroecosystems.

The advantages of *Epichloë* endophyte for host grasses are predominantly observed in areas or in years of severe drought and high insect damage. This may show the preference of sowing endophyte-infected tall fescue and ryegrass in drought prone areas of the world in order to extend grass plantations or rangeland rehabilitations. However, growing infected grasses under drought could be associated with higher toxic alkaloid accumulation leading to more fescue toxicosis and ryegrass staggers especially if turf cultivars are infected with common toxic endophytes.

Epichloë endophyte can improve mineral nutrition, biotic and abiotic stress tolerance and competitive ability of host grasses relative to endophyte-free counterparts, which in turn results in better agronomic performance in agroecosystems. Recent studies show that it could also have broader consequences, affecting higher trophic levels in ecosystems. However, the stability of endophyte effects over years and under different environments may not be consistent. This urges investigation on how the effects of *Epichloë* endophyte may differ between host species, regions/countries and years.

Despite the vast majority of studies focusing on drought and mineral stresses, the outcome of endophyte infection against some stressors like flood, high soil salinity, heat, cold and nutrient deficiency in ecosystems has been scarcely addressed. It is evident that a lot of variation from positive to negative impact of endophyte infection on host tolerance to biotic and abiotic stresses does exist. This is believed to originate from grass genetic background, endophyte genetics and their interactions. In a large population of grass genotypes of a given species in an ecosystem, there should be interactions from parasitism to full mutualisms and their mean effect could be neutral unless ecological constraints favor some infected grass counterparts. Therefore, there is a need to understand the environmental drivers which promote compatible combinations of endophyte-host plants resulting in superiority over non-infected or incompatible endophyte-host counterparts. As stress conditions are likely to intensify in grassland ecosystems in the future, there may be a greater reliance on endophyte infection for growth and production in ecosystems.

Grasses infected with *Epichloë* endophyte may have the potential to decrease the need for application of pesticides, help weed management and phytoremediation, and desalinization of agricultural soils. The applied consequences of *Epichloë* infection to engineer agroecosystems in these ways remain to be explored. Due to many multi-lateral interactions, selection of superior grass-endophyte combinations is difficult and deserves greater attention to develop protocols or models for rapid evaluation of variation that would be useful in agroecosystems. Also, similar to many other plant-microbe interactions, the relationship has a genetic basis and for better exploitation of this symbiosis for ecosystem purposes, the plant genes responsible for receiving or rejecting the endophytic partner should be identified.

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