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REVIEW PAPER

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DYNAMICS OF *Epichloë*-GRASS RELATIONSHIPS: AN ANALYSIS OF THE DUALISTIC NATURE

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ABSTRACT

This review provides a comprehensive discussion of *Epichloë* fungi, grass endophytes that form deeply integrated symbioses, including the taxonomy and morphology of these organisms, along with the evolution of their classification based on molecular data. The complex nature of their symbiotic interactions is detailed, ranging from mutualistic protection against biotic and abiotic stresses (e.g., drought, silicon accumulation, enhanced photosynthesis) to potential livestock toxicity resulting from alkaloid production. We present analyses of the fung's life cycles, including vertical (mutualistic) and horizontal (pathogenic) transmission, and the role of hybridization in shaping alkaloid profiles. The production of alkaloids (lolines, indole-diterpenes, ergot alkaloids, peramine) and their biological effects are discussed. We also highlight the dynamics of host specificity and coevolution. Furthermore, detection and characterization methods are presented, emphasizing molecular techniques like PCR and microsatellite analysis for rapid and precise strain identification. Finally, the significant agronomic and ecological implications of *Epichloë* endophytes are addressed, underscoring their potential in sustainable agriculture through the development of "safe" and effective strains. New topics included in the review include information on the use of modern molecular markers for rapid and precise strain identification. Furthermore, the review highlights the extended benefits of endophyte presence for the host through silicon accumulation and manipulation of photosynthesis, as well as a new approach to alkaloid biosynthesis through domain shuffling, which allows for genetically based prediction of alkaloid profiles.

Key words: alkaloids, biotic and abiotic stresses, *Epichloë*, grass endophyte

INTRODUCTION

Epichloë fungi are a group of endophytes that form complex and long-lasting symbiotic relationships with various grass species [Schardl et al. 2004, Christensen 2008]. These microscopic organisms colonize the intercellular spaces of the host plant, spending most of their life cycle within it, typically without causing visible external symptoms [Siegel et al. 1989, Clay 1990]. This hidden presence, coupled with their systemic growth throughout the plant, from leaves to seeds, indicates a highly evolved and deeply integrated symbiotic relationship. The fungus must possess sophisticated mechanisms to avoid or suppress host defense responses, enabling prolonged coexistence [Mathew et al. 2022, Zhao et al. 2025]. This deep integration is a prerequisite for the observed systemic benefits, as the fungus must be widely distributed within the plant to exert its protective effects in its tissues [Schardl 1996, Siegel and Bush 1996, Grabka et al. 2022].

The dualistic nature of *Epichloë* species is one example of biological complexity in nature, which can be considered on two main levels: ecological (their relationship with the plant) and agricultural (their impact on animals). This stems from the diversity of alkaloids they produce, which, on the one hand, have a beneficial effect on the plant by increasing its resistance to biotic and abiotic stresses (drought, salinity, heavy metals, fungal diseases,

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and pests). At the same time, others, such as ergovaline and lolitrem, are highly harmful to livestock, negatively impacting their health, productivity, and fertility.

The role of *Epichloë* fungi extends far beyond mere coexistence, making them key factors in the biological protection of grasses. They provide resistance to a range of biotic factors, such as nematodes, insects, wild animals, and grazing livestock [Bastias et al. 2017, Karpyn Esqueda et al. 2017, Caradus 2023], as well as to abiotic stresses, including drought [Schardl 1996, Rodriguez and Redman 2008, Nagabhyru et al. 2022]. Their influence extends to modulating grassland ecosystems, impacting both aboveground and belowground processes [Vikuk et al. 2019, Wang et al. 2020, Wang et al. 2024]. However, despite these significant benefits, the interactions of *Epichloë* with grasses present a complex challenge. Some fungal strains produce compounds that are toxic to livestock, creating a paradox where the same symbiont can be both immensely beneficial and potentially harmful [Caradus and Johnson 2020, Fredell et al. 2025]. This duality is a primary driver of research and commercial applications, aiming to harness the benefits while minimizing the risks. If a biological agent has both highly beneficial and highly detrimental effects, it poses a complex challenge for agricultural management. This inherent tension demands a scientific approach focused on understanding the mechanisms of both benefits and harms, leading to strategies for selective breeding or engineering of "safe" and effective strains [Schardl and Leuchtmann 2005, Saikkonen et al. 2016, Pozo et al. 2021].

The aim of this review is to comprehensively discuss *Epichloë* fungi, grass endophytes that form deeply integrated symbioses, taking into account their taxonomy, evolution, morphology, and the complex nature of their symbiotic interactions. Furthermore, it explores additional host benefits beyond alkaloid production, the dynamics of host specificity and coevolution, and methods for detecting and characterizing these fungi. The paper also delves into the significant agronomic and ecological implications of *Epichloë* endophytes, including their potential applications in sustainable agriculture.

TAXONOMY, MORPHOLOGY AND CHARACTERIZATION OF *Epichloë* FUNGI

Epichloë fungi belong to the phylum *Ascomycota*, order *Hypocreales,* and family *Clavicipitaceae* [White 1994, 1997, White and Reddy 1998, Du et al. 2024]. These are grass endophytes that spend most of their life cycle within the intercellular spaces of their host plant, typically without causing visible external symptoms [Siegel et al. 1985, White and Cole 1985, Gams et al. 1990, Wilson 1995]. In laboratory conditions, on solid media, their morphological characteristics such as colony color, diameter, and growth rate are thoroughly examined, often by measuring colony diameter using the "cross" method [Wang et al. 2025].

The endophytes are relatively slow-growing fungi and require specific nutrient-rich media for successful isolation and characterization. The most common universal medium recommended for initial isolation and observing colony morphology is potato dextrose agar (PDA). However often to stimulate sporulation in certain strains is using cornmeal agar (CMA). Additionally is using also malt extract agar (MEA), which provides a rich source of nitrogen and carbon, and is suitable for long-term maintenance of cultures [Stone et al. 2000].

The history of *Epichloë* taxonomy exemplifies the evolution of fungal classification, driven by advacements in molecular methods [Schardl et al. 2004]. Initially, anamorphic (asexual) grass endophytes were classified under the genus *Acremonium*, then reclassified to *Neotyphodium* [Glenn et al. 1996]. However, based on comprehensive phylogenetic reviews and DNA sequence data, such as 18S ribosomal DNA, beta-tubulin gene (*tubB*), and translation elongation factor 1-alpha gene (*tefA*), *Neotyphodium* species were re-incorporated into the genus *Epichloë* [Schardl et al. 2004, Leuchtmann et al. 2014]. This reclassification reflects a shift from classification based solely on phenotypic (morphological) characteristics to a system based on genetic and evolutionary relatedness. Traditional mycological classification often relied on observable reproductive structures. Asexual fungi (anamorphs) were grouped separately from their sexual counterparts (teleomorphs) if the sexual stage was unknown or absent. With the advent of DNA sequencing, it became possible to trace evolutionary lineages more accurately. This revealed that many *Neotyphodium* species were, in fact, asexual forms or hybrids of known *Epichloë* species. This reclassification is not merely a name change but reflects a fundamental shift in our understanding of their biological identity and evolutionary relationships, emphasizing genetic continuity over reproductive strategy. An example is the endophyte from *Ammophila breviligulata* Fern. initially identified as *Acremonium typhinum* var. *ammophilae* White and Morgan-Jones. and subsequently *Epichloë typhina* var. *ammophilae* (White and Morgan-Jones) White, which was phylogenetically identified as a member of *E. amarillans* White [Drake et al. 2018].

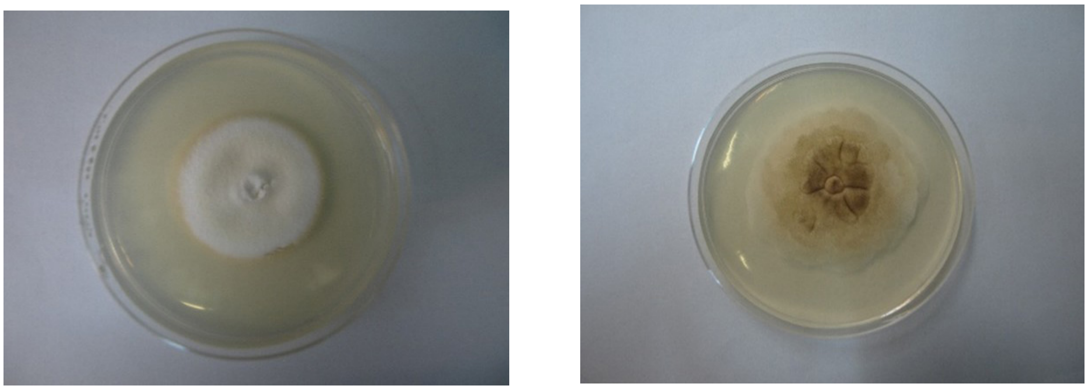
Epichloë fungi colonize a wide range of grasses, predominantly from temperate zones, encompassing numerous species. These include fescues such as meadow fescue (*Festuca pratensis* Huds.), tall fescue (*Festuca arundi-*

nacea Schreb.), red fescue (*Festuca rubra* L.), sheep fescue (*Festuca ovina* L.), hair fescue (*Festuca capillata* Lam.), and giant fescue (*Festuca gigantea* L.). These endophytes also occur in ryegrasses: perennial ryegrass (*Lolium perenne* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) [Christensen et al. 1997, Schardl et al. 2004, Scott et al. 2012, Leuchtman et al. 2014, Becker et al. 2016]. The host list further includes bluegrasses like Kentucky bluegrass (*Poa pratensis* L.) and wood bluegrass (*Poa nemoralis* L.), timothy (*Phleum pratense* L.), tufted hairgrass (*Deschampsia caespitosa* (L.) P.B.), various *Agrostis* species (e.g., redtop – *Agrostis gigantea* Roth), orchardgrass (*Dactylis glomerata* L.), and alkali grass (*Puccinellia distans* (Jacq.) Parl.). Additionally, *Epichloë* has been found in grasses such as reed grasses (*Calamagrostis arundinacea* L. (Roth.), *C. villosa* (Chaix) J.F.Gmel., *C. varia* (Schrad.) Host, *C. purpurea* (Trin.) Trin.), as well as *Bromus erectus* Huds., *B. benekenii* (Lange) Trimen, *B. ramosus* Huds., *Elymus repens* (L.), *E. tsukushiensis* Honda, *Hordelymus europaeus* (L.) Jess. ex Harz, *H. brevisubulatum* (Trin.) Link, *Leymus chinensis* (Trin.), soft brome (*Holcus mollis* L.), and velvet grass (*H. lanatus* L.) [Clay and Schardl 2002, Schardl et al. 2004, Schardl 2010, Luna-Fontalvo et al. 2025].

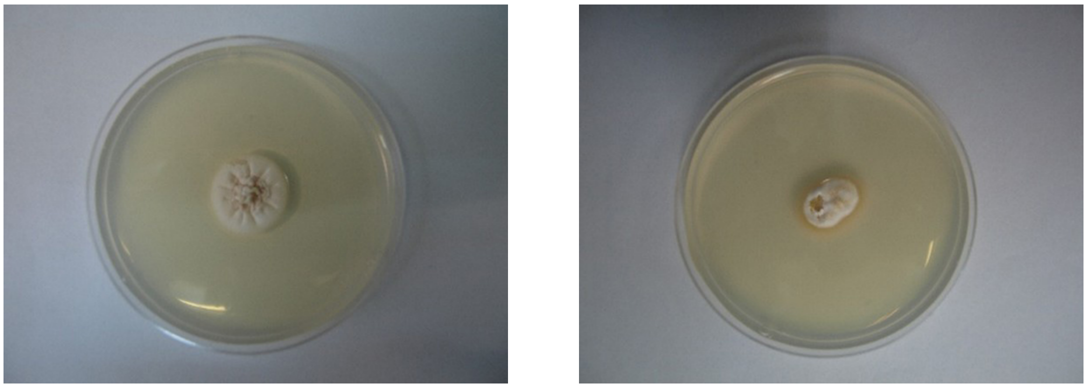
The morphological characteristics of *Epichloë* fungi in culture, such as colony color, diameter, growth rate (Fig. 1), conidia size, and conidiogenous cell length, exhibit significant interspecific variability [Schardl et al. 2004, Leuchtman et al. 2014].

Although these properties are not sufficient for definitive taxonomic grouping, they are crucial for initial isolation, identification, and understanding of the physiological diversity within the genus [Schardl et al. 2004]. Wang et al. [2025] observed clear differences in growth rate and conidia sizes among various *Epichloë* species, which is utilized in laboratory research for strain characterization. This means that morphological analysis, combined with molecular data, provides a more complete picture for strain identification and characterization, aiding in the selection of specific strains for agricultural applications or in understanding their ecological niches [Leuchtman et al. 2014, Du et al. 2024]. This also highlights the need for a multi-faceted approach to identification.

Figure 1. Appearance of pure *Epichloë* cultures isolated from individual grass species after 3 weeks of incubation (photo B. Wiewióra)



Festuca rubra *Festuca pratensis*



Lolium perenne *Poa pratensis*

METHODS OF DETECTION AND CHARACTERIZATION

Detecting and characterizing *Epichloë* fungi in their host plants has evolved from traditional techniques to advanced molecular methods, allowing for precise and rapid identification. Early in-plant detection methods included histological staining, which allowed visualization of fungal hyphae [Latch and Christensen 1985]. Immunological assays, such as ELISA and tissue-print immunoblot, were also employed [Gwinn et al. 1991, Hill et al. 2002]. More recently, PCR-based methods have become dominant for endophyte detection and quantification, both in culture and in plants, due to their speed, sensitivity, and specificity [Doss and Welty 1995, Dombrowski et al. 2006, Sharma et al. 2020]. The evolution from histological staining and immunological assays to sophisticated PCR-based methods, especially microsatellite analysis, marks a critical shift toward precise diagnostics in *Epichloë* research. This allows for rapid, sensitive, and highly specific strain identification, which is paramount for risk management in agriculture (e.g., identifying toxic strains) and for targeted breeding programs. Older methods were often slower, less specific, or required culturing the fungus first, which could take weeks. In contrast, molecular methods can directly detect the fungus in plant tissue, providing quick results. This speed and precision are essential in agricultural settings where rapid identification of endophyte strains (and their associated alkaloid profiles) is necessary for decisions regarding livestock grazing or seed certification [Takach and Young 2014].

- Randomly Amplified Polymorphic DNA (RAPD): this is a PCR-based method used for detection and quantification [Moon et al. 2000].
- Microsatellite Loci Analysis (Microsatellite-based PCR Fingerprinting): this method leverages the polymorphic properties of microsatellite loci (short tandem repeats) with the speed and sensitivity of PCR. It enables precise determination of amplified product size through automated analysis using fluorochrome-labeled primers and laser scanners. A reference database of allele sizes can be created, allowing for distinguishing endophyte groups at the level of known isozyme groups. The assay is specific for *Epichloë* DNA in the plant, even in complex mixtures of plant DNA, and can be expedited with specific DNA extraction methods [Takach et al. 2012]. Certain loci (e.g., B11, B9, B10) are particularly informative, allowing for differentiation of multiple patterns and identification of hybrid endophytes due to the presence of multiple alleles. Multiplex PCR can be used to amplify multiple loci simultaneously, saving reagents and time. Automated analysis provides high detection sensitivity, safer handling, faster data collection, simplified analysis of hybrid endophytes, and data generation as DNA fragment size for interlaboratory comparisons. The ability to combine genetic characterization (e.g., presence/absence of alkaloid biosynthesis genes via PCR) with precise strain identification (via microsatellite analysis) allows for a predictive approach to toxidrome risk assessment [Schardl et al. 2013b]. This means that farmers and breeders can know the potential toxicity of a grass-endophyte combination without having to wait for symptoms in livestock, facilitating proactive management. If a specific *Epichloë* strain can be identified and its genetic potential for alkaloid production is known, it's possible to predict whether it will cause "fescue toxicosis" or "ryegrass staggers" before any animals are affected, which has enormous economic and animal welfare benefits.
- Phylogenetic Analysis: the phylogenetic position of *Epichloë* isolates can be investigated using specific genes such as *tefA* and *actG* or *tubB* [Craven et al. 2001].

While Microsatellite Loci Analysis enables the identification of hybrid endophytes through the presence of multiple alleles, the rapid evolution of genomics has introduced tools that provide unparalleled resolution. Especially crucial for analyzing the complex, heteroploid genomes often found in *Epichloë* species Long-Read Sequencing (LRS), Pangenome Frameworks or SNP Assays and Digital PCR (dPCR) [Schreiber et al. 2024, Huggett and Whale 2013]. Equally important is comprehensive alkaloid profiling based on multiplex LC-MS/MS (liquid chromatography with tandem mass spectrometry) or LC-HRMS (liquid chromatography with high-resolution mass spectrometry) [Rudolph et al. 2018, Berry et al. 2019, Vassiliadis et al. 2019]. These modern genomic and chemical tools deliver the speed, precision, and comprehensive resolution required to manage the economic and animal welfare aspects of the grass-endophyte symbiosis effectively today.

Epichloë-GRASS SYMBIOSIS: A CONTINUUM OF INTERACTIONS

The nature of the symbiosis between *Epichloë* and grasses is variable, ranging from antagonism to mutualism, depending on prevailing selective pressures, environmental conditions, and the specific genotypes of the fungus and host. Their mode of interaction is highly dependent on the surrounding situation, environment, or specific conditions [Saikkonen et al. 1998, Faeth and Sullivan 2003, Saikkonen et al. 2016]. Antagonistic aspects often arise from the fungus's sexual cycle, during which it produces stromata that can cause abortion of host inflorescences, leading to "choke disease," which is considered the pathogenic end of the continuum [Saikkonen et al. 1998, von Cräutlein et al. 2021]. Conversely, mutualistic aspects include protective fungal alkaloids and other benefits for systematically infected plants. Host benefits are largely associated with defensive mutualism, resulting from bioactive fungal-derived alkaloids [Bush et al. 1997, Schardl et al. 2004, Cadus and Johnson 2020].

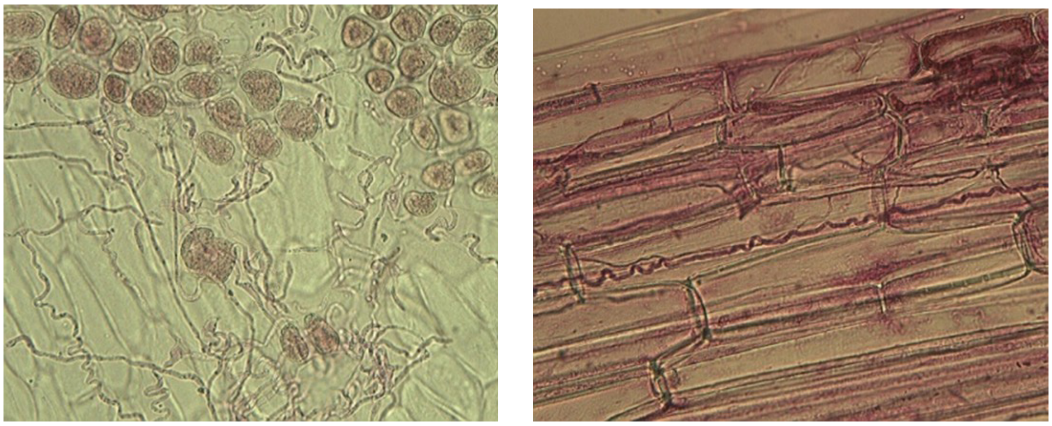
The symbiosis is characterized by a high degree of integration, involving the systemic growth of the fungus in the host's aerial parts, including leaf sheaths, inflorescences, ovules, and seed embryos [Clay 1990]. The fungus grows intercellularly within the host tissues. This intimate relationship requires the fungus to adapt to gain access to the host plant's interior, likely by suppressing the plant's recognition and defense responses that would normally halt harmful fungal infections [Saikkonen et al. 1998]. The necessity for the fungus to suppress host recognition and defense responses indicates a continuous process of reciprocal evolutionary adaptation [Schardl et al. 2012]. The host attempts to detect and defend itself against fungal invaders, while the endophyte evolves mechanisms to evade or modulate these defenses to establish a stable, asymptomatic infection. This suggests a complex molecular dialogue between host and symbiont [Lee et al. 2021]. Plants have developed sophisticated immune systems to detect pathogens [Jones and Dangl 2006]. For an endophyte to live asymptomatically within a plant, it must either be "invisible" to the plant's defense mechanisms or actively suppress them [Saikkonen et al. 1998, Redman et al. 2002, Rodriguez et al. 2009]. This is not a passive process; it represents a finely tuned co-adaptation where the fungus has evolved specific strategies to bypass or disarm host immunity, enabling the establishment of a symbiotic rather than a pathogenic state, which underscores the molecular sophistication of this interaction [Redman et al. 2002, Jones and Dangl 2006, Dodds and Rathjen 2010, Schardl et al. 2012].

Epichloë fungi exhibit various life cycles, categorized primarily by transmission methods, reflecting a fundamental evolutionary trade-off.

Vertical transmission (asexual forms)

These forms, formerly known as *Neotyphodium*, are obligate endophytes [Schardl et al. 2004, Leuchtman et al. 2014]. They are clonally and vertically transmitted from the mother plant to its progeny via host seeds [Caradus and Johnson 2020]. The fungus penetrates the developing ovule and eventually the embryo and endosperm of mature seeds [Philipson and Christey 1986, Rodriguez et al. 2009] (Fig. 2). Asexual *Epichloë* species typically do not produce sexual spores (hymenium) and rarely produce asexual spores (conidia), except under axenic culture conditions in the laboratory [Clay 1990, Schardl et al. 2004]. Many asexual strains are interspecific hybrids, often resulting from hybridization events between two different *Epichloë* species. They usually possess heteroploid genomes [Charlton et al. 2014]. This mode of transmission is associated with high host specificity and often favors the formation of beneficial (mutualistic) associations [Zabalgogeazcoa 2008]. If a microorganism survival relies solely on the host's ability to reproduce and transmit it through seeds, then any trait that harms host reproduction (such as inflorescence strangulation) would be strongly eliminated. This creates strong evolutionary pressure for mutualism [Saikkonen et al. 1998].

Figure 2. Characteristic hyphae in the seed (left) and in the plant (right) (photo B. Wiewióra)

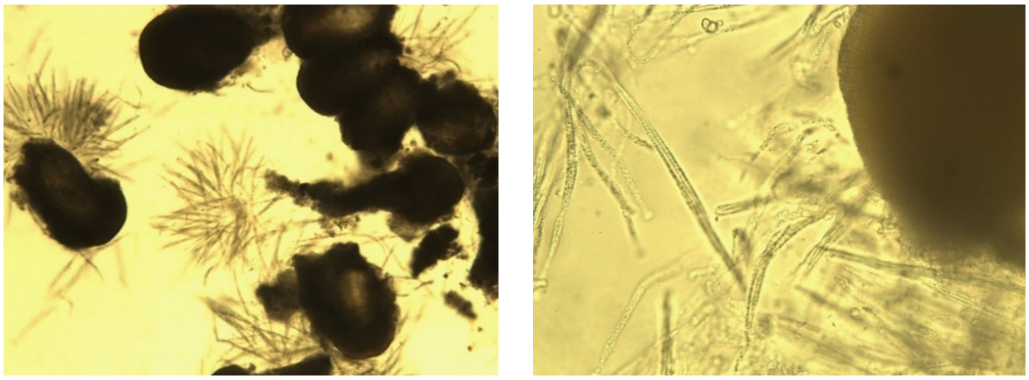


Horizontal transmission (sexual forms)

Sexual *Epichloë* species produce fruiting bodies called stromata, which encircle the immature host inflorescence and halt its development, causing "choke disease" [White 1997] (Fig. 3). These are transmitted horizontally via sexual spores (ascospores) [Schardl et al. 2004] (Fig. 4). However, this is not the only possibility of horizontal transmission, because Wiewióra et al. [2015] found in their work that horizontal transmission of mycelia is possible also through the transfer of hyphae during mowing and trampling of grass.

Figure 3. Stromata on meadow timothy (left) and sheep's fescue (right) (photo: B. Wiewióra)

Figure 4. Asci and ascospores of *Epichloë* sp. ×100 (left) and ×400 (right) (photo: B. Wiewióra)



Fertilization requires the dissemination of spermatia (male gametes) onto unfertilized stromata of the opposite mating type [Clay and Schardl 2002]. This process is vectored by phylogenetically distinct flies from the family *Anthomyiidae* (e.g., *Botanophila* spp., formerly *Phorbia* spp.), which are attracted by fungal volatile compounds, feed on the perithecial tissue containing spermatial spores, and lay their eggs on the stromata [Bultman et al. 1998]. Horizontally transmitted *Epichloë* fungi typically possess haploid genomes [Schardl and Craven 2003]. This strategy can be considered pathogenic due to host castration [Chung et al. 1997]. Conversely, if a microorganism can spread independently via spores, it has less "stake" in the reproductive success of a single host, allowing for more exploitative or pathogenic strategies. This distinction is crucial for understanding the ecological roles and agricultural applications of different *Epichloë* strains.

Pleiotropic species

Some *Epichloë* species e.g. *E. typhina* exhibit a mixed strategy, undergoing both sexual (horizontal) and asexual (vertical) cycles [Schardl et al. 2004]. The frequent occurrence of asexual *Epichloë* strains as interspecific hybrids is a significant evolutionary mechanism [Tsai et al. 1994]. This hybridization allows for the combination of genetic material from different sexual species, potentially leading to new alkaloid profiles and host associations that are highly beneficial and vertically transmissible, without the "cost" of the sexual cycle (choke disease). Sexual reproduction allows for genetic recombination and diversity. If asexual strains are hybrids, they gain genetic diversity from their sexual ancestors without needing to undergo their own sexual cycle. This can lead to new gene combinations, including those responsible for alkaloid biosynthesis, potentially creating strains with unique defensive capabilities that are highly adapted for mutualistic, vertical transmission. This explains how "novel endophytes" with desired traits can arise and be selected for agricultural applications.

ALKALOID PRODUCTION AND BIOLOGICAL EFFECTS

Epichloë species are known for producing a range of secondary metabolites, primarily alkaloids, which are crucial for defensive mutualism with their grass hosts. The production of these alkaloids depends on the specific *Epichloë* species and can be modified by environmental conditions and host genotype. Alkaloid diversity is largely determined by the presence or absence of specific alkaloid biosynthesis genes [Moore et al. 2015]. However, research has also shown that structural diversity in secondary metabolites, such as pyrrolopyrazines, can arise through mechanisms like nonenzymatic cyclization and domain shuffling in nonribosomal peptide synthetases [Berry et al. 2019]. This allows for predicting synthesized alkaloids based on genetic studies, which is much more efficient than chemical analysis alone. This genetic understanding is fundamental for developing "novel endophytes" that produce beneficial, insect-deterring compounds without vertebrate toxins, and serves as a powerful selection tool.

There are four main classes of bioactive alkaloids (Table 1):

- lolines (saturated 1-aminopyrrolizidines)
- indole-diterpenes
- ergot alkaloids
- peramine (pyrrolopyrazine alkaloid).

The differentiation of alkaloid classes into those primarily toxic to insects (lolines, peramine) and those also toxic to vertebrates (ergot alkaloids, indole-diterpenes) suggests an evolutionary specialization of defense mechanisms (Table 2). This means that *Epichloë* has evolved to counteract a wide range of herbivores, but with varying degrees of specificity and collateral impact. If a single defense mechanism worked against all herbivores, there would be no need for multiple alkaloid classes. The existence of distinct classes with different target specificities (insects vs. vertebrates) also presents a challenge for agriculture: selecting strains that maintain a broad spectrum of insect defense while eliminating or minimizing "collateral damage" to livestock, which are unintended targets of some of these defense mechanisms.

Table 1. Classes of alkaloids produced by *Epichloë* endophytes and their biological effects on herbivores and livestock

Alkaloid class	Examples	Effects on livestock	Insecticidal properties
Lolines	N-formylloline (NFL), N-acetylloline (NAL), N-acetylnorloline (NANL)	no known mammalian toxicity	repellent and toxic to insects (e.g., Argentine stem weevil, black cutworm, aphids, scarab larvae)
Indole-diterpenes	lolitrem B, epoxyjanthitrems, paxilline, terpendoles	"ryegrass staggers" – neurotoxins, tremors, affect muscle coordination, respiration, heart rate	anti-insect properties (e.g., Argentine stem weevil, root aphids)
Ergot alkaloids	ergovaline, ergotamine, ergonovine, agroclavine, lysergic acid	"fescue toxicosis" – inability to regulate temperature, vasoconstriction, heat stress, reduced prolactin	anti-insect properties (e.g., black cutworm, Argentine stem weevil)
Peramine	peramine, pyrrolopyrazine	no known mammalian toxicity	insect repellent (e.g., Argentine stem weevil, aphids)

Alkaloid profiles can differ even within the same *Epichloë* species, which is due to the genetic diversity of the alkaloid gene clusters, and the host plant can moderately influence the level of alkaloid production by the endophyte, although to a lesser extent than the genetics of the fungus [Talamantes et al. 2025]. Furthermore, the distribution of alkaloids within the plant can vary and doesn't always correlate with the fungal hyphae's distribution [Tian et al. 2020]. Alkaloid concentrations are usually highest in the seeds. In the shoots/roots, the distribution can be different, e.g. peramine and lolitrem B concentrations are often higher in the shoots, while ergovaline may be more evenly distributed [Vassiliadis et al. 2023, Realini et al. 2024].

Table 2. *Epichloë* species in grasses and the alkaloids they produce

Species of *Epichloë*	Host plant	Alkaloids produced	Reference
E. amarillans	*Agrostis hyemalis*	NANL (N-acetylnorloline), PER (peramine)	Schardl et al. 2013a
E. amarillans	*Agrostis hyemalis*	ERV (ergovaline), PER (peramine)	Schardl et al. 2013a
E. baconii	*Calamagrostis villosa*	PER (peramine)	Schardl et al. 2013a
E. brachyelytri	*Brachyelytrum erectum*	CC (chanoclavine), AcAP (1-acetamidopyrrolizidine), PER (peramine)	Schardl et al. 2013a
E. bromicola	*Bromus erectus*	no alkaloids detected	Schardl et al. 2013a
E. bromicola	*Bromus tomentellus*, *Melica persica*, *Thinopyrum intermedium*	LOL (loline alkaloids)	Schardl et al. 2025
E. coenophiala	*Festuca arundinaceum*	Ergovaline alkaloids (e.g. ergovaline), peramine, lolines	Emile et al. 2000 Vazquez de Aldana et al. 2001 Żurek et al. 2010 Wiewióra 2011 Leuchtmann et al. 2014
E. elymi	*Elymus virginicus*	CC (chanoclavine), PER (peramine)	Schardl et al. 2013a
E. festucae	*Festuca rubra*, *Lolium giganteum*	ERV (ergovaline), NFL (N-formylloline)	Leuchtmannn et al. 2000 Żurek et al. 2010 Wiewióra 2011 Schardl et al. 2013a
E. festucae	*Festuca trachyphylla*	ERV (ergovaline), LTB (lolitrem B), PER (peramine)	Schardl et al. 2013a
E. festucae var. lolii	*Lolium perenne*	LTB (lolitrem B), PER (peramine), ergovaline alkaloids	Oliveira et al. 1997 Cagaš et al. 1999, Żurek et al. 2010 Wiewióra 2011, Schardl et al. 2013a
E. funkii	*Achnatherum robustum*	CC (chanoclavine), TD (tremorgenic indole-diterpenes), PER (peramine)	Schardl et al. 2013a
E. gansuensis	*Achnatherum inebrians*	PAX (paxilin)	Schardl et al. 2013a
E. gansuensis var. *inebrians*	*Achnatherum inebrians*	LAH (lysergic acid α-hydroxyethylamide), ergonovine, ergine	Schardl et al. 2013a
E. glyceriae	*Glyceria striata*	ERV (ergovaline), AcAP (1-acetamidopirolizidine)	Schardl et al. 2013a
E. mollis	*Holcus mollis*	ERV (ergovaline), PER (peramine)	Schardl et al. 2013a
E. poae	*Poa nemoralis*	no alkaloids detected	Schardl et al. 2013a
E. poae	*Poa nemoralis*	ERV (ergovaline)	Schardl et al. 2013a

Species of *Epichloë*	Host plant	Alkaloids produced	Reference
E. typhina	*Lolium perenne*	PER (peramine)	Schardl et al. 2013a
E. uncinata	*Festuca pratensis*	loline compounds	Cagaš et al. 1999 Żurek et al. 2010 Wiewióra 2011 Vikuk et al. 2019
Epichloë sp.	*Festuca ovina* agg.	indole-diterpene intermediates	Vikuk et al. 2019
Epichloë sp.	*Achnatherum robustum*	ergonovine, ergine	Guerre 2015
Epichloë clarkii	*Holcus lanatus*	No data	Leuchtmann et al. 2014

It's worth noting that alkaloid expression levels can be quantitatively modified by environmental factors. They are strongly expressed in the plant, but at very low levels or not at all in axenic cultures (without the presence of other organisms) [Aniszewski 2007].

HOST BENEFITS BEYOND ALKALOID PRODUCTION

Beyond the well-documented chemical defense mechanisms, the symbiosis with *Epichloë* provides grass hosts with a range of other physiological and developmental benefits, increasing their overall fitness and resilience.

Epichloë endophytes can provide host plants with resistance to various abiotic stresses, including drought tolerance, salt tolerance, and waterlogging [Rodriguez and Redman 2008, Decunta et al. 2021]. A particularly interesting mechanism is silicon (Si) accumulation. The presence of *Epichloë* significantly increases silicon concentration in the leaves of host grasses; for example, in tall fescue this increase is at least 31%, and in perennial ryegrass it is 47% for specific strains [Cibils-Stewart et al. 2020]. This increase is independent of plant growth and positively correlates with endophyte colonization. Potential mechanisms for increased Si uptake may include increased transpiration rates by symbionts, a direct impact on plant aquaporins, or alterations to endogenous host defense pathways (e.g., the jasmonic acid pathway) that promote Si uptake. Morphological changes, such as an increased number of vascular bundles, may also contribute to this phenomenon. Interestingly, silicon supply can, in turn, increase *Epichloë* colonization (e.g., by over 60% in tall fescue), likely by creating additional niches or increasing carbon availability for the fungus. The discovery that *Epichloë* increases host silicon accumulation, and silicon in turn enhances *Epichloë* colonization, reveals a sophisticated positive feedback loop. This suggests a deeper level of mutualism where the fungus not only provides direct benefits but also actively modifies the host physiological environment in a way that optimizes its own growth and survival within the plant [Ma and Yamaji 2006]. This goes beyond a simple "fungus gives, plant receives" model. If the fungus helps the plant acquire a resource (Si) beneficial for the plant structural integrity and defense, and this resource also benefits fungal colonization, it indicates a coevolutionary mechanism that strengthens the symbiosis itself. This is a more complex interaction than just the production of defensive compounds; it is about active shaping the host's internal environment for the mutual benefit of both partners, making the symbiosis more resilient and effective.

The fungal endophyte *Epichloë typhina* significantly improves the growth, PSII photochemistry, and carbon assimilation efficiency of its host, orchardgrass (*Dactylis glomerata*) [Rozpądek et al. 2015]. These mechanisms include increased biomass, increased electron transport rate (ETR), photochemical quenching coefficient (qL), and effective quantum yield of PSII (Y(II)). *E. typhina* also enhances net photosynthesis and carboxylation rates and lowers the CO_2 compensation point. Increased stomatal conductance is also observed. Furthermore, in *Epichloë*-colonized plants, a higher total chlorophyll concentration (approximately 33% increase) and a twofold increase in chlorophyll b concentration were found. The increased abundance of PSII (D1, Lhcb3) and PSI (PsaC, Lhca2) proteins indicates a higher photosynthetic capacity. The elevated activity of NADPH-malate dehydrogenase (NADPH-MDH)—a sixfold increase—helps in thylakoid lumen deacidification and maintaining an appropriate ATP/NADPH ratio, potentially providing additional energy resources for the plant or its fungal partner [Rozpądek et al. 2015]. The detailed mechanisms by which *Epichloë typhina* enhances photosynthesis, including increased chlorophyll b, PSII/PSI proteins, and NADPH-MDH activity, indicate that the fungus is not merely a passive recipient of host photosynthates. Instead, it appears to actively influence host metabolism to increase overall carbon assimilation. This suggests a sophisticated level of metabolic integration and manipulation by the endophyte to ensure a greater supply of resources, not only for itself but also to enhance the host's overall fitness. For an endophyte that relies on host-assimilated carbon, increasing host photosynthetic efficiency is a highly beneficial strategy. The specific increases in light-harvesting complexes and enzymes involved in carbon assimilation and energy distribution suggest a targeted metabolic adaptation of the plant, likely induced by the fungus. This is a powerful example of how a symbiont can directly improve its host's fundamental energy production capacity, supporting the "increased energy demand" of the symbiosis while simultaneously leading to higher host biomass [Rozpądek et al. 2015, Sarkar et al. 2021]. The positive role of endophytes in disease resistance is demonstrated also in studies by Fardella [2024], whose results allowed the identification of a specific antifungal protein called Efe-AfpA. Expression studies confirmed the key role of this protein in the mechanism of endophyte-induced resistance. The characterization of a number of other proteins homologous to Efe-AfpA in various fungal species indicates the existence of enormous, as yet untapped potential in the development of modern methods of plant disease control.

Beyond specific stress tolerances, *Epichloë* symbioses generally enhance the host plant's fitness, leading to increased growth, vigor, and competitive advantage [Clay 1990, Schardl et al. 2004]. Selected asexual *Epichloë* strains can increase plant biomass and seed yield [Malinowski and Belesky 2019, Gundel et al. 2020].

Despite enormous progress in research on the symbiosis between grasses and fungi of the genus *Epichloë,* the influence of these microorganisms on the dynamics of plant communities and soil processes remains one of the least understood areas of endophyte ecology. Most analyses to date have focused on direct host protection from biotic (herbivory) and abiotic (drought) stress, neglecting subtle but crucial indirect mechanisms. However, *Epichloë* has been shown to indirectly affect the rhizosphere. Although this fungus does not physically colonize root tissues, it radically alters its biochemistry by inducing changes in the transport of assimilates to roots, which influences the quantity and chemical composition of root exudates. These changes may selectively promote specific groups of rhizosphere bacteria, altering the structure of the soil microbiome [Zhong et al. 2022]. One of the most controversial aspects is the impact of *Epichloë* on mycorrhizal fungi (AMF). Some studies suggest that aboveground endophytes may compete with AMF for carbon resources, while others indicate synergy in increasing drought resistance [Omacini et al. 2012, Li et al. 2018].

HOST SPECIFICITY AND COEVOLUTIONARY DYNAMICS

The fitness of the *Epichloë* symbiont and its grass host are intimately linked, presumably leading to their coevolution towards specialization and mutually beneficial cooperation [Clay and Schardl 2002, Schardl et al. 2004]. This coevolution involves reciprocal interactions that often lead to specialization. The symbiosis is highly integrated, involving the mutual exploitation and manipulation of morphological, physiological, and life cycle traits to enhance the fitness of the "symbiote" [Clay 1988].

Host specificity is primarily maintained through the vertical transmission of the microbial partner from the host plant to its progeny via seeds [Clay 1990]. This ensures a stable interaction between the fungal genotype and the host lineage. A loss of horizontal spread (horizontal transmission) is often associated with increased genetic host specificity [Moon et al. 2002, Moon et al. 2004, Kuldau and Bacon 2008]. The intimate relationship requires the fungus to adapt to gain access to the host plant interior, likely by suppressing the host recognition and defense responses that would normally halt harmful fungal infections [Schardl et al. 2004]. The clear mention of "genetic incompatibility" limiting endophyte-grass associations is a crucial observation for both ecological understanding and agricultural applications [Christensen 1995]. This means that simply having the right fungal and grass species is insufficient; specific genotypes must be compatible for a stable and beneficial symbiosis to form and persist. This explains the challenges associated with artificially infecting non-hosts and the need for careful strain-to-cultivar matching in commercial products. If every *Epichloë* strain could infect every grass, the system would be much simpler. The concept of genetic incompatibility suggests that there are specific recognition or compatibility genes on both sides that must match for the symbiosis to succeed. This is a significant barrier to horizontal gene transfer or a broad host range, reinforcing the idea of specialization. For agriculture, it means that developing new *Epichloë*-grass associations is not trivial and requires extensive screening and breeding to find compatible partners. Asexual species, often formed through hybridization, have an even more limited host range and have coevolved towards one or a few grass species, forming a mutualistic relationship [Tsai et al. 1994].

The benefits for *Epichloë* species and their grass hosts are rarely symmetrical, and the symbiosis can range from antagonistic to mutualistic [Clay and Schardl 2002]. Conflicting selective pressures likely lead to their destabilization. For example, when pleiotropic and antagonistic *Epichloë* species enter their sexual cycle, they can eliminate host seed production [Chung et al. 1997]. The benefits from endophytes depend on the fungal and host genotypes and environmental conditions [Cheplick and Faeth 2009].

AGRONOMIC AND ECOLOGICAL IMPLICATIONS

The *Epichloë* symbiosis significantly impacts agricultural practices and natural ecosystems, evidenced by the successful commercialization of novel endophytes, their potential in cereal crops, and the challenges and risks that require careful management and regulation. Asexual, symbiotic *Epichloë* strains are widely used and commercialized in agriculture, most notably in the United States and New Zealand, to reduce livestock toxicity while maintaining pest deterrence in pastures by incorporating selected 'novel endophyte' strains into grass cultivars [Fletcher and Easton 2000, Malinowski et al. 2000]. These selected *Epichloë* strains have become key components of many pasture ecosystems, especially in New Zealand, where an estimated 90% of perennial ryegrass sold contains a selected endophyte strain [Johnson and Caradus 2019, Caradus et al. 2021]. The development and widespread commercialization of "novel endophytes" represent a paradigm shift in agricultural pest management. Instead of relying solely on external chemical applications, the plant itself becomes a "chemical factory," producing its own internal defense mechanisms [Popay and Bonos 2005]. This move towards integrated biological solutions has significant implications for reducing environmental impact and addressing issues like pesticide resistance. The history of *Epichloë*, especially the "novel endophyte" approach, demonstrates a shift toward leveraging internal biological systems. By selecting strains that produce beneficial but non-toxic compounds, agriculture can achieve pest control from within the plant, reducing the need for synthetic chemicals. This approach is much more sustainable and environmentally friendly, aligning perfectly with modern agricultural goals. Moreover, this technology has a significant economic impact; for example, the AR37 endophyte strain alone has contributed approximately NZD 3.6 billion to New Zealand's economy over 20 years [Thom et al. 2012].

BENEFITS OF THE *Epichloë*-GRASS SYMBIOSIS

The symbiosis between *Epichloë* fungi and grasses is an example of a mutually beneficial relationship that offers a number of significant benefits to the host plant and, consequently, to agriculture and the environment. These endophytes, which live within plant tissues without causing disease symptoms, have become the subject of intense research due to their potential as a natural plant protection agent and a factor in improving plant immunity. One key advantage of this symbiosis is its role in biological pest control. *Epichloë* endophytes produce alkaloids that are toxic to herbivorous insects, effectively reducing insect predation [Popay et al. 1995, Popay et al. 2003]. This acts as a built-in, natural defense system for the plant, minimizing the need for chemical pesticides. This not only reduces costs for farmers but also significantly reduces environmental impacts, promoting more sustainable agricultural practices. Furthermore, plants colonized by *Epichloë* exhibit increased resistance to common foliar diseases of grasses and cereals. This includes reduced susceptibility to rust, powdery mildew, and leaf spot, as confirmed by studies [Lee et al. 2021, Card et al. 2021]. This natural defense reduces yield losses due to disease and reduces the need for fungicide intervention. The *Epichloë*-grass symbiosis also contributes to increased plant tolerance to abiotic stresses. Endophytic plants are more resistant to drought, flooding, and potentially other negative effects of climate change [Rodriguez and Redman 2008, Decunta et al. 2021]. The ability to survive in harsh environmental conditions is extremely valuable in the face of increasing climatic challenges and weather variability. Furthermore, the presence of *Epichloë* endophytes is associated with improved vigor, biomass, and seed yield in colonized plants [Malinowski and Belesky 2000]. This translates into healthier and more productive crops, which directly translates into greater agricultural productivity. In the broader context, this symbiosis offers potentially lower costs for farmers and a reduced environmental impact. Reduced demand for chemical pesticides and fertilizers [Johnson et al. 2013] not only lowers farm operating expenses but also supports biodiversity and soil health, contributing to a more ecologically sustainable food system.

CHALLENGES AND RISKS ASSOCIATED WITH *Epichloë* – GRASS SYMBIOSIS

Despite its numerous benefits, the symbiosis between *Epichloë* fungi and grasses also presents certain challenges and potential threats that require attention, especially in the context of their commercial exploitation.

One of the most significant problems is the production of alkaloids by some *Epichloë* strains, which are toxic to livestock. Examples of such substances include ergovaline and lolitrem, which can cause serious conditions such as fescue toxicosis and ryegrass staggers [Dougherty et al. 1991, Bush et al. 1993]. These conditions can lead to significant economic losses in livestock farming, manifesting as decreased weight gain, reduced milk production, reproductive problems, and even death. For this reason, agriculture strives to use grass varieties colonized by *Epichloë* strains which do not produce alkaloids harmful to animals but still provide protection against pests. Another aspect requiring caution is the risk of horizontal fungal transfer when commercially releasing *Epichloë* strains capable of sexual reproduction into the environment. Ascospores produced by these strains can be carried long distances by wind, potentially spreading the fungus to wild grass species or previously uninfected croplands [Glenn et al. 1996]. Such uncontrolled transfer can have unpredictable ecological consequences, including altering the species composition of ecosystems or increasing the stress tolerance of wild grasses, which could affect their invasiveness. Furthermore, the presence of *Epichloë* endophytes in grasses can negatively impact invertebrate biodiversity. Studies have shown that grasses infected with *Epichloë* can reduce populations of beneficial soil organisms, such as earthworms [Omacini et al. 2012]. This is likely due to toxic alkaloids, which can leach into the soil or be consumed by invertebrates feeding on plants or organic matter. Reducing the number of these organisms can disrupt natural ecological processes, such as nutrient cycling and soil aeration. Finally, the increased host plant fitness provided by the symbiosis with *Epichloë* could potentially make grass species invasive in natural or agricultural environments [Clay 1990, Chen et al. 2022]. Plants with *Epichloë* are more competitive, cope better with harsh conditions, and are more resistant to pests and diseases. As a result, they can displace native grass species, disrupting local ecosystems and leading to biodiversity loss. This risk is particularly significant when new *Epichloë* strains are introduced into regions where they do not occur naturally. Understanding these potential threats is crucial for the responsible use of the *Epichloë*-grass symbiosis in agricultural practices and environmental protection.

CONCLUSIONS

Fungi of the genus *Epichloë* are an exceptionally important component of grassland ecosystems and agricultural systems, offering a complex network of interactions that range from mutualistic to antagonistic. Their ability to systematically colonize grasses and produce bioactive alkaloids provides significant benefits to their hosts, such as resistance to pests, diseases, and abiotic stresses, making them valuable tools in sustainable agriculture. Advances in taxonomy, driven by the development of molecular techniques and precise detection methods, have enabled a deeper understanding of their biology and potential.

However, the duality of their action – the ability to produce toxins harmful to livestock (e.g. ergovaline, lolitrem) as well as those that increase resistance to environmental stresses (e.g. peramine) – underscores the need continuous research on this group of fungi and for careful management and stringent regulatory frameworks. The development of “novel endophytes,” which provide benefits to plants without harming animals, represents a significant advancement. The future of *Epichloë* research focuses on further harnessing their potential, including expanding their applications to cereal crops and utilizing genetic engineering to tailor alkaloid profiles. These research directions are crucial for addressing global challenges related to food security, climate change, and reducing reliance on chemical plant protection products, while ensuring the responsible and ethical implementation of these powerful biological solutions.

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