Fungal endophytes for sustainable crop production

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Abstract

This mini-review highlights the importance of endophytic fungi for sustainable agriculture and horticulture production. Fungal endophytes play a key role in habitat adaptation of plants resulting in improved plant performance and plant protection against biotic and abiotic stresses. They encode a vast variety of novel secondary metabolites including volatile organic compounds. In addition to protecting plants against pathogens and pests, selected fungal endophytes have been used to remove animal toxicities associated with fungal endophytes in temperate grasses, to create corn and rice plants that are tolerant to a range of biotic and abiotic stresses, and for improved management of post-harvest control.
We argue that practices used in plant breeding, seed treatments, and agriculture, often caused by poor knowledge of the importance of fungal endophytes, are among the reasons for the loss of fungal endophyte diversity in domesticated plants and also accounts for the reduced effectiveness of some endophyte strains to confer plant benefits. We provide recommendations on how to mitigate against these negative impacts in modern agriculture.

Keywords

1. Introduction

1.1. Definition of 'endophyte'

We define endophytes based on the definition proposed by Hallmann and co-authors (Hallmann *et al*., 1997), as "those microorganisms that can be isolated from surface-disinfected plant tissue or extracted from within the plant, and that do not visibly harm the plant". However, this definition is not perfect as, firstly, it does not account for endophytes that are unculturable and, secondly, it is not always easy to assess phytopathogenicity and distinguish latent pathogens from endophytes, particularly for non-culturable fungi that are part of the microbiome community (see Hyde & Soytong, 2008; Card *et al*., 2015; Hardoim *et al*., 2015; Mercado-Blanco, 2015; Card *et al*., 2016 for a discussion of the term 'endophyte'). Fungal endophytes exhibit a range of symbiotic relationships with their hosts as well as

various lifestyles; for example, some of these interactions can be mutualistic in which the long-term relationship is beneficial to both partners (reviewed in Card *et al*., 2016). Additionally, some endophytes may only exhibit a mutualistic interaction for one plant species, but not for another (Hardoim *et al*., 2015). However the scientific community generally agrees that endophytes are microorganisms that can be detected inside healthy plant tissues and are asymptomatic. Finally, for experimental purposes, in order to verify that a microorganism has an endophytic lifestyle, it must be demonstrated that it can be successfully re-introduced into disinfected seedlings as judged by microscopy, thereby fulfilling Koch's postulates (Hyde & Soytong, 2008).

1.2. Aim of this review

After introducing some basic aspects of fungal endophytes, the primary aim of this paper is to provide insights into the means by which fungal endophytes can be effectively used for improving growth, health, and adaptation of plants to stress conditions. Examples of fungal endophytes which are used in, or have promise for, agriculture and horticulture, will be presented. While several reviews have described aspects of fungal endophytes (Saikkonen *et al*., 1998; Strobel & Daisy, 2003; Schulz & Boyle, 2005; Arnold, 2007; Rodriguez *et al*., 2008; Aly *et al.,* 2011; Porras-Alfaro & Bayman, 2011; Friesen, 2013; Johnson *et al*., 2013a) none have focused on the combined aspects of how:
(i) fungal endophytes and their secondary metabolites improve plant health, plant performance and plant adaptation by using selected fungal endophytes;
(ii) the commercialization of fungal endophytes and their products can be successfully achieved, and;
(iii) fungal endophytes and/or their plant-beneficial activities could have been lost or reduced from cultivated plants through plant breeding and use of fungicides and how this can be prevented in the future.

1.3. Occurrence

Within all plants there is a diverse microbial community comprising bacterial, archaeal, fungal, and protistic taxa (Hardoim *et al*., 2015). Blackwell (2011) discusses the classification, discovery and identification of fungi as well as estimations of the magnitude of fungal diversity present on earth which ranges from the conservative 1.5 million species by Hawksworth (2001) to the newer estimates of 5.1 million based on high throughput sequencing methods. Researchers estimate there are at least 1 million endophytic fungal species (Strobel & Daisy 2003; Ganley *et al*., 2004); a number of such magnitude signifies the importance of fungal endophytes as crucial constituents of fungal biodiversity. Endophytic fungi are predominantly ascomycetes that appear to be ubiquitous in nature as they have been recovered from plants adapted to a wide range of ecosystems that include hot deserts, Arctic tundra, mangroves, temperate and tropical forests, grasslands and savannahs, and croplands (Arnold 2007; Arnold and Lutzoni 2007; Arnold 2008). Present in all the major lineages of land plants, they are common to mosses and other nonvascular plants, ferns and other seedless plants, conifers, and flowering plants (Arnold 2007). Our understanding of fungal endophyte

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diversity across large geographical areas is still developing but endophyte diversity in plants has been observed to decrease from the tropics to northern boreal forests (Arnold 2007; Arnold and Lutzoni 2007). Furthermore, endophyte communities from higher latitudes are characterized by relatively few fungal species, whereas tropical endophyte assemblages are dominated by a small number of classes but a very large number of different endophytic species (Arnold *et al*., 2000; Arnold & Lutzoni 2007). The spectrum of fungi within a plant varies in space, time and function. For example, leaves, stems, and roots of alfalfa plants are colonized by distinct fungi that produce different ranges of secondary metabolites (Weber & Anke, 2006; Porras-Alfaro & Bayman, 2011). A compelling feature of fungal endophytes is their exceptional diversity both at a global scale and at the scale of individual leaves, plants, and locations (Arnold, 2008).

While acknowledging that the majority, if not all plants contain endophytes, many remain not studied for their endophytic diversity or function. However, the exponential increase in the use of endophytes for improving plant adaption, which has featured in recent reviews (Johnson *et al*., 2013a; Kivlin *et al*., 2013; Porras-Alfaro & Bayman, 2011; Hardoim *et al*., 2015; Card *et al*., 2016) indicates a growing recognition by the scientific community of the potential to exploit novel endophytes for the improvement of crop production.

1.4. Functional grouping of endophytic fungi

The differentiation of fungal endophytes into functional groups based on various criteria is a useful framework for clarifying the unique symbiotic and ecological functions of endophytic fungi and to aid researchers in answering fundamental biological questions about these organisms. Rodriguez *et al*. (2009) described different functional groups of endophytic fungi based on their phylogeny and life history traits. Class 1 endophytes are defined as the Claviciptaceous endophytes (including *Balansia* spp. and *Epichloë* spp.). The *Epichloë* species form systemic associations with the aboveground tissues of grasses and are one of the most economically important examples of plant-endophyte interactions (Johnson *et al*., 2013a). The non-clavicipitaceous types are further separated into three sub-classes, Class 2, 3 and 4. The diverse Class 2 endophytes encompass both Ascomycota and a few Basdiomycota (the Dikarya). The most distinctive feature is their ability to colonize roots, stems and leaves and the formation of extensive plant infections (Rodriguez *et al*., 2009). Endophytes from Class 3 are extremely diverse and form highly localized infections in aboveground tissues, such as in the leaves of tropical trees and nonvascular and vascular plants (Rodriguez *et al*., 2009). The Class 4 endophytes are also referred to as the dark-septate endophytes (DSE) (Rodriguez *et al*., 2009) and these facultative biotrophic fungi colonize plant roots and have the distinguishing feature of having melanized dark septate hyphae (Jumpponen & Trappe 1998; Jumpponen 2001, 2003). We recommend that an additional class is needed to recognize the endophytic entomopathogenic fungi as symptomless endophytes of plants that have the unique ability to infect and colonize insects (Quesada Moraga *et al*., 2014; Vidal & Jaber, 2015).

1.5. Transmission

The means of propagation or the transmission routes of many endophytic fungi remain to be determined, however, they can be transmitted either horizontally or vertically. The *Epichloë* species are one example where the endophyte can be propagated via both routes as long as the ability to produce ascospores on the host plant is retained. One of the well-studied examples of vertical transmission via seed has been described for the asexual *Epichloë* species. These fungi cannot produce reproductive structures on their hosts and are naturally propagated by growing into the embryo of a developing seed and, subsequently, as the seed germinates, hyphae colonize the young seedling (Philipson & Christey, 1986). Moving vertically transmitted *Epichloë* endophytes from one plant to another can be manipulated in the laboratory (Latch & Christensen, 1985) enabling the artificial inoculation of grasses for developing desirable grass-endophyte combinations for possible commercialization purposes (Johnson *et al*., 2013a). Another type of vertical transmission occurs via the seed coat and has been reported for Class 2 endophytes (Rodriguez *et al*., 2009). A well-known example is that of *Curvularia protuberata* which confers high soil temperature tolerance to its tropical grass host in a geothermal habitat (Redman *et al*., 2002).

In the case of horizontal transmission, propagation is usually dependent on the reproductive structures of the endophyte, such as spores, that move by wind or rain dispersal, or are moved by a vector, from plant to plant. This can occur via the soil, through air movement, or by vectors, e.g. insects. For the Class 2 and 3 endophytes, plant colonization likely occurs via infection structures such as appresoria or directly via the hyphal penetration of plant tissues (Ernst et al. 2003; Gao & Mendgen, 2006). The horizontal transmission of the sexual species of *Epichloë* (Class 1) via ascospores has been well documented (reviewed in Schardl *et al*., 2004) and in contrast to the asexual *Epichloë*, their presence is not asymptomatic but forms external reproductive structures (stromata) on flowering tillers, preventing floral development and seed production (choke disease). These sexual species are obligately out-crossing (heterothallic) and a third mutualist partner, a fly species from the genus *Botanophila*, acts as the vector to transfer spermatia (conidia – mitotic spores) of one mating type to the stroma of the opposite mating type (Schardl *et al*., 2004). Ascospores are produced if mating partners are compatible and are ejected into the air and wind-dispersed for mediating contagious infections (Chung & Schardl, 1997; Brem & Leuchtmann, 1999). Interestingly, the choice of transmission route that the sexual *Epichloë* species selects for propagion, influences how beneficial or antagonistic the symbiotic outcome is for the host plant due to the direct effect the endophyte life cycle has on host reproduction.

1.6. Role in the ecosystem

Fungal endophytes play crucial roles in ecosystems by protecting plants against many biotic and abiotic stresses, increasing their resilience, and helping plants to adapt to new habitats (Strobel & Daisy, 2003; Schulz, 2006; Rodriguez *et al*., 2008; Aly *et al*., 2011; Friesen, 2013). Biotic stresses from which endophytes can provide protection include plant pathogens, insects and nematodes. Abiotic stresses include nutrient limitation, drought, salination, and extreme pH values and temperatures.   In return, plants provide spatial structure, protection from

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desiccation, nutrients and, in the case of vertical transmission, dissemination to the next generation of hosts (Schulz, 2006; Aly *et al*., 2011). Endophytes may also play a role in the ecosystem by affecting plant growth through antagonistic fungal-fungal interactions. An example is the interaction between the pathogen *Ustilago maydis* and the endophyte *Fusarium verticillioides* within their shared plant host (maize (*Zea mays*)), whereby the endophyte is capable of reducing the rate of pathogen growth, possibly by secreting metabolites that break down plant compounds that limit *U. maydis* growth (Rodriguez Estrada *et al.*, 2012).

A further role of some fungal endophytes in ecosystems may be to initiate the biological degradation of a dead or dying host plant which starts the process of nutrient recycling (Strobel & Daisy, 2003; Zhang *et al*., 2006; Vega *et al*. 2010; Aly *et al*., 2011; Boberg *et al.,* 2011). A survey of grass endophytes by Vázquez de Aldana *et al*., (2013) shows that the dominate endophyte taxa of grasses are also found as common airborne fungi. They speculate that the transition from an endophyte to a saprobe requires sporulation after host senescence for some of these species to complete their life cycle. Being established in tissues, endophytes have immediate access to plant nutrients available during plant senescence (Rodriguez *et al*. 2008; Aly *et al*., 2011).

2. Fungal endophytes for improving plant performance

Fungal endophytes are well known to contribute plant fitness benefits, enabling adaptation of the plant host to biotic and abiotic stresses (reviewed in Rodriguez & Redman, 2008; Aly *et al*., 2011; Franken 2012; Johnson *et al*., 2013; Johnson *et al*., 2014; Card *et al*., 2016). These fungi have diverse secondary metabolites (Tan & Zou, 2001; Gunatilaka 2006), some of which are bioactive compounds expressed as defensive weapons to protect the host plant from pests and diseases but also as metabolites for specific interactions and communications with the plant host. Habitat-adapted symbiosis, a term coined by Rodriguez et al. (2008), defines how certain fungal endophytes (Class 2 types) adapt to stress in a habitat-specific manner under different environmental conditions. The molecular and biochemical mechanisms behind how this habitat-adapted symbiotic interaction results in plant tolerance to high stress is unknown. Endophyte-enhanced plant growth promotion is another conferred beneficial trait that at least for the root-colonizing endophyte *Piriformospora indica*, is likely achieved through enhanced nutrient uptake and translocation, and by the modulation of phytohormones involved in growth and development (Johnson *et al*., 2014).

2.1 Fungal endophytes for conferring pest and disease resistance

Epichloë endophytes (Class 1) are found in some temperate grass species and their primary symbiotically important contribution is to produce bioactive metabolites in the host plant which act as deterrents to pests and herbivores. The common toxic endophyte, *Epichloë festucae* var. *lolii*, originally present in perennial ryegrass and introduced into New Zealand from Europe, produces three known major secondary metabolites:

(i) Lolitrem B (Fig. 1.A) is a neurotoxin now known to cause ryegrass staggers (Gallagher *et al*., 1981, 1982; Fletcher 1993; Fletcher *et al.,* 1999).
(ii) Ergovaline is a metabolite that causes vaso-constriction (Oliver 2005) resulting in heat stress (Fletcher 1993; Fletcher *et al*., 1999) and in tall fescue it causes the condition referred to as fescue toxicosis (Hoveland, 1993; Bacon, 1995).
(iii) Peramine (Fig. 1.B) is an insect feeding deterrent (Rowan *et al*., 1990; Rowan *et al*., 1993).

While endophyte-free grass is not toxic to animals, it can become susceptible to insect pests. A novel *Epichloë* strain (AR1) was introduced 15 years ago which produces neither lolitrem B nor ergovaline but the production of peramine provides resistance to a major insect pest - Argentine stem weevil (*Listronotus bonariensis*) (Johnson *et al*., 2013a). Later discoveries of other novel *Epichloë* endophyte strains identified another alkaloid present in endophytes, the epoxy-janthitrems (Finch *et al*., 2012) which are a type of indole diterpene found only in perennial ryegrass infected with strain AR37 (Tapper & Lane, 2004). AR37-infected grasses have broad pesticidal effects against five of the six major pasture pests present in New Zealand, however, the role of the epoxy-janthitrem compounds in insect resistance has not yet been fully elucidated (Johnson *et al.* 2013a). Because of the advantage these novel endophytes provide through improving ryegrass persistence but without causing significant detrimental animal effects, the uptake by farmers has been rapid (Caradus *et al*., 2013). A more comprehensive story on the importance of secondary metabolites present in *Epichloë* strains, and how they have been applied, can be found in Johnson *et al*. (2013a).

Epichloë endophytes have not only received worldwide recognition for their importance in pasture systems in delivering both economic and sustainable agricultural solutions, but they also represent an excellent model system for investigating various aspects of endophyte biology in addition to exploring both the ecological implications and the molecular basis of the symbiosis. *Epichloë festucae* in particular can be cultured on axenic media and is amenable to molecular functional studies. A growing number of studies characterizing *E. festucae* mutants have revealed that the maintenance of both reactive oxygen species (Tanaka *et al*., 2006, 2008; Takemoto *et al*., 2006, 2011) and iron homeostasis (Johnson *et al*., 2013b) are essential factors for maintaining healthy mutualistic interactions with grasses.

The root-colonizing facultative endophyte *Piriformospora indica* forms beneficial symbioses with crop plants. It has the potential for use in agriculture, horticulture and floriculture (for reviews see Oelmüller *et al*., 2009; Franken, 2012; Qiang *et al*., 2012; Ansari *et al*., 2013; Oberwinkler *et al*., 2013; Unnikumar *et al*., 2013; Johnson *et al*., 2014; Gill *et al*., 2016). *P. indica* was originally isolated by Verma *et al*. (1998) from the rhizosphere of several xerophytic plants located in the Indian Thar dessert, India. This filamentous fungus belongs to the order Sebacinales in Basidiomycota (Weiss *et al*., 2004) and confers numerous host benefits, such as the promotion of plant growth, particularly in conditions of nutrient stress, and confers tolerance to a wide range of abiotic stresses (drought, temperature extremes, water, salinity, heavy metals), as well as biotic (root and foliar pathogens) stresses (see above reviews for details). Original inoculation experiments showed the ability of *P. indica* to

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colonize plant roots (Verma *et al*., 1998) and to date, *P. indica* is renowned for its broad host range, forming symbiotic root interactions with both mono- and dicotyledonous plants, including the agriculturally important barley (*Hordeum vulgare*), and the model plants, tobacco (*Nicotiana tabacum*) and *Arabidopsis thaliana* (Johnson *et al*., 2014).

Plants colonized with *P. indica* show an enhanced tolerance to various root and foliar pathogens (see Table 2, Johnson *et al*., 2014 for a list of plant diseases controlled by *P. indica*). The potential of *P. indica* as a biocontrol solution to manage various root diseases in different crop species such as maize (Kumar *et al*., 2009), tomato (Fakhro et al., 2010), wheat (Rabiey *et al*., 2015; Serfling *et al*., 2007) and barley (Waller *et al*., 2005) is evident. Systemic resistance to different foliar pathogens via *P. indica*-interactions has also been demonstrated (Qiang et al., 2012). Fakhro *et al*. (2010) studied the effects of inoculation of tomato with *P. indica* and observed that in soil, *P. indica* colonizes the roots of tomato, increases the biomass of the leaves by up to 20%, and reduces the disease severity caused by *Verticillium dahliae*, the causal agent of Verticillium wilt, by more than 30%. In hydroponics, *P. indica* increases fresh fruit biomass of tomato by up to 100%, results in higher numbers of fruits, and increases the dry matter content by up to 20%. Moreover, at high light densities it represses Pepino Mosaic Virus which has been found widely in tomato greenhouses in many European countries, in Morocco, South and North America, and in China (Fakhro *et al*., 2010).

Many other fungal endophytes have been shown to protect host plants from pathogenic fungi. The endophyte *Epicoccum nigrum* isolated from sugarcane is especially known for its biocontrol activity against pathogens, such as *Sclerotinia sclerotiorum* in sunflower, *Pythium* in cotton, phytoplasma bacteria in apple and *Monilinia* spp. in peaches and nectarines (Fávaro *et al*., 2012)**.** Murphy *et al*. (2015b) isolated fungal endophytes from wild barley plants (*Hordeum murinum* subsp. *murinum* L.) and demonstrated that some were able to successfully suppress the activity of seed-borne fungal infections on seeds of cultivated barley. The tested seeds harbored some of the most devastating pathogens of barley, including species of *Cochiolobus*, *Fusarium*, *Pyrenophora* and *Rhynchosporium*. Two fungal endophytic isolates, including a *Penicillium brevicompactum* strain, gave 100 % suppression of the infections. In addition to suppressing seedborne pathogens, this strain also suppressed growth of the soil-borne pathogen *Gaeumannomyces graminis* var. *tritici* (Murphy *et al*., 2015b).

Fungal endophytes isolated from healthy *Theobroma cacao* tissues have been screened *in vitro* for antagonism against major pathogens of cacao including *Moniliophthora roreri* (causing frosty pod rot), *Phytophthora palmivora* (causing black pod rot) and *Moniliophthora perniciosa* (causes witches broom) (Mejía *et al*., 2008). Field trials have assessed the effects of these endophytic fungi and have shown that treatment with *Colletotrichum gloeosporioides* significantly decreased pod loss due to black pod rot and that treatment with *Clonostachys rosea* reduced the incidence of sporulating lesions of *M. roreri* on cacao pods (Mejía *et al*., 2008).

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Lastly, fungal entomopathogens are an important class of endophytes to consider for their anti-insect biocontrol properties (Quesada Moraga *et al*., 2014). They have a unique ability to infect and colonize insects affecting insect survival and reproduction and many, if not all, have the ability to colonize plants (Vidal & Jaber, 2015). Interestingly, most of the commercially produced entomopathogenic fungi can be endophytic for part of their life cycle and include the species of *Beauveria*, *Metarrhizium*, *Lecanicillium*, and *Isaria* (Vidal & Jaber, 2015). None of these commercial biocontrol products have made use of their endophytic mode of action however, but at least one such product is able to colonize plants endophytically (reviewed in Vidal & Jaber, 2015). Wagner and Lewis (2000) first described in detail the penetration and colonization of plant tissues by the entomopathogenic fungus, *Beauveria bassiana*. Vidal & Jaber (2015) further review the colonization of plants by entomopathogenic fungi and discuss the variability and effectiveness in colonization rates observed when using different strain/cultivar combinations. Entomopathogenic fungi are effective against several root-feeding insects belonging to different orders, such as the root mealy bug, *Cataenococcus ensete*, the sugar beet root maggot, *Tetanops myopaeformis*, the diabroticine rootworms, and the white grubs *Melolontha melolontha* and *Popillia japonica* (Li *et al*., 2000). The coffee berry borer (*Hypothenemus hampei*) is the most devastating pest of coffee throughout the world. Vega *et al*. (2010) collected fungal endophytes of coffee plants from Hawaii, Colombia, Mexico, and Puerto Rico. They found various genera of fungal entomopathogens, including *Acremonium, Beauveria, Cladosporium, Clonostachys,* and *Paecilomyces. B. bassiana* and *Clonostachys rosea* appeared to be pathogenic to the coffee berry borer (Vega *et al*., 2010). These fungal entomopathogenic endophytes also play a role in soil nitrogen cycling.   Behie *et al*., (2012) tested the ability of *M. robertsii* to translocate insect-derived nitrogen to plants. They injected waxmoth larvae with 15N-labeled nitrogen and placed the labelled insects on haricot bean (*Phaseolus vulgaris*) and switchgrass (*Panicum virgatum*) and observed incorporation of 15N into amino acids of the plant, but only in the presence of *M. robertsii*.

2.2 Fungal endophytes for conferring abiotic stress, including impacts of climate change
Fitness benefits conferred by mutualistic fungal endophytes contribute to, or are responsible for, plant adaptation to not only biotic stresses but also abiotic stress by increasing tolerance to drought and water stress, as well as tolerance to high temperature and high salinity (Aly *et al*., 2011).

Habitat-adapted stress tolerance of plants conferred by habitat-specific symbionts is an intriguing phenomena hypothesized to be responsible for the establishment of plants in high stress environments (Rodriguez *et al*., 2008). A large number of Class 2 endophytes have been found to be capable of habitat-adaption providing tolerance to habitat-specific selective pressures such as soil pH, temperature and salinity (Rodriguez *et al*., 2009). Rodriguez et al. (2008) demonstrated that grass species adapted to coastal and geothermal habitats harbor symbiotic fungal endophytes that provide salinity and heat tolerance, respectively. *Leymus mollis* (dunegrass) plants from several coastal beach habitats in the USA were shown to be symbiotic with the endophyte *Fusarium culmorum* which, when inoculated into salt-sensitive non-coastal plants, conferred salt tolerance to those plants (Rodriguez *et al*., 2008). Similar

experiments were carried out to demonstrate that endophytic fungi aid heat tolerance. The tropical panic grass, *Dichanthelium lanuginosum*, found growing in geothermal soils in Yellowstone National Park forms a mutualistic symbiotic interaction with the Class 2 endophyte, *Curvularia protuberata* which confers heat tolerance (Redman *et al*., 2002). When root zones were heated up to 65°C, symbiotic plants infected with the endophyte tolerated and survived the heat but separately, neither the fungus nor the plant alone were capable of growing at soil temperatures above 38°C (Márquez *et al*., 2007). This relationship however is more complex as it was discovered that the endophyte harbors a virus in a tripartite mutualistic symbiosis and that the virus is an essential partner for bestowing heat tolerance to host plants, whereas virus-free endophytes were incapable of conferring this trait (Márquez *et al*., 2007). These habitat-specific symbionts also have a broad host range and have been shown to be able to confer habitat-specific tolerance to other plant species. For example, an endophyte from coastal plants can colonize the model monocot, rice, and confer salt tolerance, whereas, endophytes from other habitats (agricultural, coastal and geothermal), can confer disease resistance but not salt or heat tolerance to the model dicot tomato (Rodriguez *et al*., 2008). Interestingly, all these endophytes conferred drought tolerance to plants regardless of the habitat of origin (Rodriguez *et al*., 2008).

Decreased water availability and increased soil salinization is a growing challenge to crop growth in many parts of the world (Egamberdieva *et al*., 2008; Egamberdieva & Lugtenberg, 2014) and this problem is expected to increase due to climate change effects. The ability of fungal endophytes to confer stress tolerance to plants may provide a novel strategy for mitigating the impacts of global climate change on agricultural plant communities (Rodriguez *et al*., 2008). Redman *et al*. (2011) showed that some Class 2 fungal endophytes can confer salt and drought tolerance to two commercial rice varieties which were not adapted to these stresses. Moreover, these endophytes reduced water consumption by 20–30% while increasing growth rate, reproductive yield, and biomass of greenhouse grown plants. They also conferred cold tolerance to growth chamber and greenhouse grown plants. These findings indicate that the incorporation of fungal symbionts may be a useful strategy in both mitigating impacts of climate change on major crops and expanding agricultural production onto marginal lands (Redman *et al*., 2011).

Turning to endophytes that have the potential to promote plant growth, *P. indica* (introduced in section 2.1) is known for its promotion of vegetative growth, early flowering, seed setting and seed germination which has been repeatedly observed with species from various plant families (Franken, 2012). An extensive set of phytohormones and phytohormone signaling networks seem to be involved in mediating plant growth promotional activities which lead to increased early root growth promotion and finally to greater biomass. The extent of growth promotion is typically around 50%, but significant variation exists, due in part to variability in the environmental and experimental conditions in which the plants are grown. Considerable research has been carried out on the mechanism of salt stress tolerance conferred to plants colonized with *P. indica* and this trait has been demonstrated for barley as well as wheat, rice and tobacco and involves the induction of a high antioxidant environment for the

detoxification of reactive oxygen species and an enhanced photosynthetic efficiency (Johnson *et al*., 2014).

P. indica's wide host benefits as well as its amenability for fundamental biological studies are attractive properties for research and its potential to transform the productivity of agricultural crops sustainably is exciting. With respect to barley, *P. indica*-interactions have shown enhanced grain yield (Waller et al., 2005; Murphy *et al*., 2014) and at low temperatures with higher nutrient input, *P. indica* also appeared to trigger flowering earlier and still increase grain yield. These results indicate that *P. indica* could be developed as an effective crop treatment in low temperature-stressed barley and may have the potential to increase crop yield under colder growing environments on the proviso that adequate nutrients are supplied (Murphy *et al*., 2014). However, despite all of these described benefits Franken (2012) states that it may be difficult to place *P. indica* on the market because (i) the fungus was first isolated in India and is protected by patent in many countries (international publication number – WO 99/29177) making any commercial prospect reliant on the patent owner to manufacturing it or licensing it for manufacture, and (ii) it sometimes has unexplained negative effects on plant growth. Nonetheless, a powder formulation of *P. indica* has been developed under the trade name "ROOTONIC" and is currently deployed in field trials in India (Varma *et al*., 2013; Shrivastava & Varma 2014).

There are also other endophytes of barley that have the potential to be advantageous in agricultural settings. Murphy *et al*. (2015a) isolated endophytic fungi from wild barley (*Hordeum murinum* subsp. *murinum* L.) and in addition to various other biotic benefits previously described (in section 2.1), at least one of these isolates was able to increase grain yield in a nutrient-deficient barley cultivar (by up to 29 %). The greatest impact one of these endophytic isolates had on grain yield and shoot dry weight was achieved under the lowest nutrient input. Such strains may therefore be able to help by reducing fertilizer inputs while maintaining acceptable yields.

Continuing with the theme of improving plant performance, the Class 4 or DSE root-colonizing fungi, that are characterized by their darkly melanized septa, are an interesting group of endophytes that are present in a wide range of terrestrial ecosystems, but are especially common in polar and alpine habitats (Rodriguez *et al*., 2009). In these cold- and water-stressed environments, arbuscular mycorrhizal fungi, the typical mutualists of grass roots at lower altitudes and latitudes, are essentially absent (Newsham, 2011) and it has been suggested that DSE species might act as surrogate mycorrhiza in these habitats (Bledsoe *et al*., 1990). Inoculation of monocotyledonous and dicotyledonous plant species with these fungi increases root and shoot biomass, and shoot nitrogen and phosphorous contents. Meta-analyses performed by Newsham (2011) on data from 18 research articles, in which plants had been inoculated with DSE species indicated that they can enhance plant performance under controlled conditions, particularly when most of the nitrogen is available in an organic form such as proteins, peptides and amino acids in the rhizosphere. Plants apparently do not benefit from DSE species when roots can readily access inorganic nitrogen (Newsham, 2011).

2.3 Fungal endophytes reducing animal health and welfare issues

The most intensively studied agricultural fungal endophyte associations are those formed between mutualistic asexual *Epichloë* endophytes of the family *Clavicipitaceae* (including the formerly classified anamorphic genus *Neotyphodium* (Leuchtmann *et al.*, 2014) and the major pasture grass species in the *Festuca* and *Lolium* genera within the tribe *Poeae* (Johnson *et al.*, 2013a). These asexual species do not manifest symptoms of disease and are characterized by their vertical transmission of hyphae via host seeds.

The grazing of ryegrass and/or fescue pastures is common-place for dairy, beef and sheep production in New Zealand, parts of Australia, parts of South America, and the USA. Sheep and cattle grazing on perennial ryegrass and tall fescue infected with the common-toxic (also known as wild-type) strains of *Epichloë* can develop either a neuromuscular condition known as ryegrass staggers (Fig. 1.A) (Cunningham & Hartley, 1959), or a restriction of blood flow also associated with heat stress and often designated as fescue toxicosis (Aiken *et al.*, 2007), respectively. In Australia, the collective condition is referred to as perennial ryegrass toxicosis which can result in sheep deaths estimated to be in the hundreds of thousands in some years (Reed & Lean, 2005). It was discovered that in all three countries the condition has been caused by infection with common-toxic strains of *Epichloë* fungal endophytes (Johnson *et al.*, 2013a).

The obvious solution was to remove the offending *Neotyphodium* endophyte (now reclassified as asexual *Epichloë* species) from ryegrass and fescue (Latch & Christensen 1982). Indeed, grazing on endophyte-free grass did not cause the disease anymore but a new problem appeared. In New Zealand, ryegrass persistence was seriously affected by damage caused by invertebrate pests, especially by the Argentine stem weevil (*Listronotus bonariensis*) (Prestidge *et al*. 1982) (Fig. 1.B). In the USA, the persistence of tall fescue was also impacted (Hopkins & Alison 2006). In both regions this issue was solved by introducing selected beneficial asexual *Epichloë* strains that do not cause animal health and welfare issues but still protect against insect pests and abiotic stresses (Johnson *et al.*, 2013a; Young et al. 2013; Bouton *et al.*, 2000; Thompson *et al.*, 2001). In subsequent investigations, the chemical basis of these phenomena was discovered as explained in section 2.1. As a result asexual *Epichloë* strains have been identified that produce animal safe bioactives which are still capable of providing a deterrence to insects pests ensuring plant persistence.

3. Secondary Metabolites produced by fungal endophytes

3.1. Fungal endophytes as a source of bioactive natural products

Due to the extraordinary biodiversity of fungal endophytes, they provide a largely untapped opportunity to discover novel natural products with unique chemical structures that have been optimized by (co-)evolution with higher plants. The recent development of screening technologies has revealed the great potential of fungal endophytes for producing novel biologically active compounds with promising medicinal or agricultural applications (Zhang

et al., 2006; Aly *et al*., 2011; Wu *et al*., 2015a). These molecules can play an important role in communication between organisms, in plant protection, and plant adaptation to habitat and environmental changes. For reasons of safety and environmental problems, many synthetic agricultural agents have been, and will be, removed from the market. Secondary metabolites produced by fungal endophytes provide novel opportunities to control pests and pathogens (Strobel & Daisy, 2003).

Overall, there is much to be gained from a better understanding of endophyte produced bioactives, some of which have been found to be novel anticancer agents, antibiotics, biofuels ("Mycodiesel"; see Strobel, 2015) and antioxidants. Discovering plants that harbor these microbes capable of producing novel bioactive metabolites is the first step. In subsequent sections 3.3, 3.4, and 3.5, we will present examples of fungal endophytes and their useful secondary metabolites, but restrict discussion to those fungal secondary metabolites which have promise for, or are already being used in, agriculture and horticulture.

3.2. Discovering plants with microbial bioactive metabolites

Schulz (2001) described that certain microbial metabolites are characteristic of certain biotopes, on both an environmental and a taxonomic level. This study indicated that organisms living in unique biotopes or habitats that are subjected to constant metabolic and environmental interactions will yield even more secondary metabolites than organisms that are not (Schulz, 2001). Therefore, the search for novel secondary metabolites should primarily center on organisms that inhabit unique biotopes.

Successful collection of plants harboring endophytes which produce novel and unique natural bioactives requires the identification of plants (i) from unique environmental settings, especially those with an unusual biology, and possessing novel strategies for survival, (ii) that have an ethnobotanical history (used by indigenous peoples) and that are related to the specific uses or applications of interest, (iii) that are endemic, that have an unusual longevity, or that have occupied a certain ancient land mass, and (iv) that grow in areas of great biodiversity (Strobel & Daisy, 2003). Expeditions to the Amazonian rain forest have led to the discovery of a wide range of secondary metabolites produced by endophytic fungi (Strobel & Strobel, 2007).

3.3. Anti-microbial Secondary Metabolites

There are many examples of secondary metabolites from endophytic fungi which are being used, or have promise to be used, against pathogens and pests of horticultural and agricultural plants. This includes fungal endophytes which produce promising but so far unidentified compounds through to well understood and described anti-microbial secondary metabolites. An overview of 230 metabolites produced by plant-associated microbial strains, including many fungal endophytes, was published by Gunatilaka (2006). Examples of some well-characterized anti-microbial secondary metabolites from endophytic fungi are presented

below in alphabetical order. Several of these compounds have been described by Strobel and Daisy (2003). The chemical structures of selected compounds are shown in Fig. 2.

Some anti-microbial compounds produced by the sugarcane endophyte *Epicoccum nigrum* (Brown *et al*., 1987) have been characterized, such as epicorazines A-B (Baute *et al*., 1978), epirodines A-B (Ikawa *et al*., 1978), flavipin (Bamford *et al*., 1961), epicoccinesA-D (Zhang *et al*., 2007), epipiridones and epicocarines (Kemami *et al*., 2007). Especially the compounds flavipin and epicorazines A-B have been associated with *Epicoccum nigrum* biocontrol activity (Brown *et al*., 1987; Madrigal *et al*., 1991; Madrigal & Melgarejo, 1995).

Ambuic acid is an antifungal and anti-oomycete agent from the endophytic fungus *Pestalotiopsis microspora,* isolates of which have been found in many of the world's rainforests. It is active against several *Fusarium* species and against *Pythium ultimum* (Li *et al*., 2001). It also appears to be a quorum sensing (as defined by Fuqua *et al*.1994) inhibitor (Gary Strobel, *personal communication*). Colletonoic acid (Bills *et al*., 2002) is a compound from a *Colletotrichum* sp., isolated from *Artemisia annua*. It shows good anti-bacterial, anti-fungal, and anti-algal activities (Hussain *et al*., 2014). Colletotric acid is a metabolite of *Colletotrichum gloeosporioides*, an endophytic fungus from *Artemisia mongolica*. It displays antimicrobial activity against bacteria as well as against the fungus *Helminthosporium sativum* (Zou *et al.*, 2000*).* Cordycepsidone A was isolated from the endophytic fungus *Cordyceps dipterigena* and has strong antifungal activity against the plant pathogenic fungus *Gibberella fujikuroi* (Varughese *et al*., 2012). Cryptocandin is a lipopeptide isolated from *Cryptosporiopsis quercina*, a fungus commonly associated with hardwood species in Europe. It is active against a number of plant-pathogenic fungi, including *Sclerotinia sclerotiorum* and *Botrytis cinerea* (Strobel *et al*., 1999). It is related to known antimycotic compounds: the echinocandins and the pneumocandins (Walsh, 1992). Cryptocin is produced by *C. quercina* (Li *et al*., 2000) and possesses the most potent activity against the fungus *Pyricularia oryzae*. It also shows strong activity against the plant-pathogenic fungi *Fusarium oxysporum*, *Geotrichum candidum*, *Rhizoctonia solarum*, and *Sclerotinia sclerotiorum,* as well as against the plant-pathogenic oomycetes *Pythium ultimum*, *Phytophtora cinnamon* and *Phytophtora citrophthora* (Li *et al*., 2000). Jesterone is produced by *Pestalotiopsis jesteri* from Papua New Guinea and was isolated from an area with moist conditions in which plant pathogens, especially oomycetes, normally thrive. As anticipated, the compound has anti-oomycete activity (Li & Strobel, 2001). Pestacin and isopestacin (Fig. 3) are anti-oxidants produced by *Pestalotiopsis microspora*, an endophyte from *Terminalia morobensis* (Strobel *et al*., 2002; Harper *et al*., 2003). Both also have anti-microbial activity (Strobel & Daisy, 2003). Phomopsichalasin is a secondary metabolite from the fungal endophyte *Phomopsis* sp. and has antibacterial activity against *Bacillus subtilis*, *Salmonella enterica*, and *Staphylococcus aureus* (Horn *et al*., 1995). Scandenin was isolated from a *Derris scandens* plant from Pakistan and has strong anti-bacterial activity against *Bacillus megaterium* and good anti-fungal and anti-algal properties (Hussain *et al*., 2015). It is remarkable that none of the anti-microbial secondary metabolites produced by these endophytic fungi has been found to be produced by bacteria which control plant diseases caused by microbes (Haas &Défago, 2005; Lugtenberg & Kamilova, 2009; Pliego *et al*., 2011).

3.4. Anti-microbial volatile compounds

Although the topic of volatile antibiotics would perfectly fit into the previous section, they have been put it under a separate heading because special techniques are required to detect these compounds. Their anti-microbial activity is detected using air contact between the volatile-producing fungus and the target pathogen (see Fig. 4). The volatile mixture can subsequently be separated and identified using a combination of chemical profiles built by LC-HRMS (liquid chromatography-high resolution mass spectrometry) or NMR (nuclear magnetic resonance) and multivariate data analysis (Wu *et al*., 2015a).

The endophytic fungus *Muscodor albus*, isolated from small branches of *Cinnamomum zeylanicum* (cinnamon tree) (Worapong *et al*., 2001) effectively inhibits and kills certain other fungi and bacteria by producing a mixture of at least 28 volatile compounds (Strobel *et al*., 2001)**.** Several individual compounds had some inhibitory effect against the test fungi and bacteria, but none was lethal. However, a strong synergistic effect was found and collectively they caused death of a broad range of plant- and human-pathogenic fungi and bacteria. Five classes of compounds were detected, namely alcohols, esters, ketones, acids and lipids. Initial studies showed that the most effective class of inhibitory compounds was the esters, of which isoamyl acetate was the most biologically active compound (Strobel *et al*., 2001). Subsequently, seven new *Muscodor albus* strains were isolated which produce various novel mixtures of volatiles. However, none of the new isolates produced any of the esters known from the original *M. albus* isolate. All together, they produce 50 different volatiles with an impressive range of biological activities, such as growth inhibition of Gram-positive and Gram-negative bacteria and of plant-pathogenic fungi and oomycetes (Ezra *et al*., 2004). Interestingly, all isolates of *Muscodor albus* isolated so far appear to make bioactive volatiles (Gary Strobel, *personal communication*).

Muscodor crispans is an endophytic fungus of *Ananas ananassoides* (wild pineapple) growing in the Bolivian Amazon Basin. The fungus produces a mixture of antifungal and antibacterial volatile organic compounds. *Pythium ultimum* appeared to be the most sensitive organism of the fungal and oomycete plant pathogens tested towards the volatile mixture of *Muscodor crispans*. Other sensitive plant fungal and oomycete pathogens include *Alternaria helianthi*, *Botrytis cinerea*, *Fusarium culmorum*, *F. oxysporum*, *Phytophthora cinnamomi* , *P. palmivora*, *Rhizoctonia solani* , *Sclerotinia sclerotiorum*, and *Verticillium dahliae*. Also the plant-pathogenic bacterium *Xanthomonas axonopodis* pv. c*itri* appeared to be sensitive (Mitchell *et al*., 2010).

Interestingly, in contrast to the situation with *M. albus*, the majority of the volatiles produced by *M. crispans* are on the US Food and Drug Administration's GRAS (Generally Regarded As Safe) list of harmless substances (see the FDA website for details: http://www.fda.gov/). For instance, no azulene or naphthalene derivatives were detected in the volatile mixture of *M. crispans*. When a mixture of the GRAS compounds produced by *M. crispans* was tested, the

results were virtually identical to those of the natural mixture. As a result this mixture was suggested to have potential utility in applications ranging from food preservation to agricultural, household and industrial uses (Mitchell *et al*., 2010).

A *Gliocladium* sp., an endophytic fungus of *Eucryphia cordifolia*, was discovered to be a volatile antibiotic producer. Of particular interest was that its most abundant volatile inhibitor was identified as [8]annulene (Fig. 2) which was formerly used as a rocket fuel and now discovered for the first time as a natural product produced by an endophytic fungus (Stinson *et al*., 2003).

The endophytic fungus *Daldinia concentrica*, isolated from an olive tree in Israel, produces at least 28 volatile organic compounds which are very promising for post-harvest control. These volatiles protect dried fruits of apricot, plum and raisin from rotting. Moreover, they protect peanuts against *Aspergillus niger*, oranges against *Penicillium digitatum*, grapes against *Botrytis cinerea*, and tomato paste against *Penicillium digitatum*. Artificial mixtures of selected volatiles have great promise for application in food industry and agriculture (David Ezra & Orna Liarzi, *personal communication*).

3.5. Anti-insect Secondary Metabolites

The association of synthetic pesticides with ecological damage has led to research to discover powerful, selective, and safe alternatives (Strobel & Daisy, 2003). Until now, biopesticides are only a small part of the pesticide market but their impact is increasing. Several endophytes are known to produce secondary metabolites that have anti-insect properties. Some of their chemical structures are shown in Fig. 5.

Nodulisporic acids (Fig. 5) are indole diterpenes which exhibit potent insecticidal properties against the larvae of the blowfly. They act by activating insect glutamate-gated chloride channels. The first nodulisporic compounds were isolated from the endophyte *Nodulisporium* sp. from the plant *Bontia daphnoides*. Since this discovery the focus is on searching for more *Nodulisporium* spp. and for other producers of more-potent nodulisporic acid analogues (Demain, 2000).

Muscodor vitigenus is an endophytic fungus of a liana from the rain forest of the Peruvian Amazon. It produces a single volatile, naphtalene (Fig. 5), which acts as an insect repellent which is the active ingredient of common mothballs. It repels the adult stage of the wheat stem sawfly *Cephus cintus*. Starch in its growth medium is crucial for naphtalene production (Daisy *et al*., 2002).

Some new benzofuran compounds (5-hydroxy-2-(1'-hydroxy-5'-methyl-4'- hexenyl) benzofuran and 5-hydroxy-2-(1'-oxo-5'-methyl-4'- hexenyl)benzofuran) were isolated from an unidentified endophytic fungus from wintergreen (*Gaultheria procumbens*). Both show toxicity to spruce budworm, and the latter one is also toxic to its larvae (Findlay *et al*., 1997).

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Peramine is a pyrrolopyrazine feeding deterrent against the insect pest Argentine stem weevil (ASW), and is produced by some *Epichloë*-grass associations (Rowan, 1993; Johnson *et al*., 2013a). Indole-diterpenes are another class of alkaloids known to be produced by *Epichloë* endophytes. Lolitrem B, which is unique to *Epichloë* is an infamous example as it is the major tremorgenic mycotoxin responsible for ryegrass staggers (Gallagher *et al*., 1984), but it also reduces the growth and development of ASW larvae (Prestidge & Gallagher 1985). As mentioned in section 2.1, epoxy-janthitrems (Fig. 5), another type of indole-diterpene, are likely to have some pesticidal effect due to the broad spectrum insect resistance of the strain AR37 which is the only *Epichloë* strain known to produce these compounds (Finch *et al*, 2012; Tapper & Lane, 2004).

Similar discoveries were made in the USA where cattle grazing endophytic tall fescue pastures contracted fescue toxicosis caused by some strains of the fungal endophyte *Epichloë coenophialum*. These strains produce the alkaloid ergovaline which is responsible for the vaso-constriction of blood vessels leading to this debilitating animal health and welfare condition (Bacon 1993, 1995). While ergovaline in its own right does have a pesticidal effect (Popay *et al*., 1990; Rowan *et al*., 1990) the associated effect of fescue toxicosis and heat stress means that ergovaline is not a useful candidate for controlling pests. However, other secondary metabolites which showed no mammalian toxicity were also discovered in this fungal endophyte host plant association. These included peramine and the lolines [of which N-formyl loline (Fig. 5) is the major one] which provide broad spectrum pest resistance (Siegel & Bush, 1994; Schardl *et al*., 2004; Johnson *et al*., 2013a; Young *et al*., 2013).

3.6. Secondary Metabolites with other plant-beneficial activities

Genomic sequencing of endophytic fungi has indicated that, despite the observation that these organisms contain gene clusters encoding certain secondary metabolites, some are not expressed under standard laboratory cultivation conditions. This raises the question as to which physiological and environmental conditions are required for endophytic fungi to express these secondary metabolites (Brakhage, 2013; Netzker *et al*., 2015). In some cases, as observed with *Epichloë,* the host genome is required for significant expression of the endophyte's secondary metabolite pathways (Schardl & Panaccione, 2005). For example, plant signaling is required to induce expression of the fungal gene clusters for lolitrem B biosynthesis by *Epichloë festucae var. lolii* so that it is highly expressed *in planta* but expression is low or undetectable in culture-grown fungal mycelia (Young *et al*., 2006)**.** The same applies to other secondary metabolites such as lolines and epoxy-janthitrems of *Epichloë* species that produce little or no compound when grown *ex planta* (Porter 1994; Tapper & Lane 2004) despite attempts to circumvent this under in culture conditions as found with loline production (Blankenship *et al*., 2001).

In other cases, endophyte secondary metabolites may be triggered by the limitation of food sources, competition with other organisms, the presence of plant components (including exudates), the presence of other fungi and bacteria as well as their metabolites, and their

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presence in plants attacked by pathogens and pests. Several examples of conditions under which the expression of secondary metabolites have been induced are published. For example, flavonoids present in root exudates of host plants induce the expression of *Rhizobium* Nod-factors (Van Brussel *et al*., 1986). Accordingly, the lack of a host stimulus in culture media may explain why the production of biomolecules by a nascent endophyte isolate is often severely attenuated through sub-culturing (Li *et al*., 1998)**.**

Because microbes in nature live in communities, growth of various fungi in co-culture with other organisms has been tested. Co-culture can indeed result in activation of silent gene clusters or in strongly increased expression (Ola *et al*., 2013; Wu *et al*., 2015b). For reviews on the effects of co-culturing, we refer to Bertrand *et al*., 2014 and Wu *et al*. 2015a. An interesting observation is that in the case of co-cultivation between *Aspergillus niger* and actinomycetes, an intimate physical interaction is required to activate silent gene clusters in *A. niger* (Schroeckh *et al*., 2009).

4. Commercialization of fungal endophytes and their products

Discoveries in the fields of fundamental microbial and plant ecology have resulted in commercial products which not only generate revenue and employment, but also provide a major contribution to food production and adaptation to environmental changes.

4.1. Commercialization of the obligate mutualistic *Epichloë* endophyte

Losses due to neuromuscular diseases and heat stress of sheep and cattle grazing on toxic temperate grasses containing asexual *Epichloë* species expressing lolitrem B or ergovaline (see section 2.3) are substantial (Johnson *et al*., 2013a). In the USA, the financial losses caused through fescue toxicosis from cattle grazing tall fescue infected with the ergovaline producing common-toxic endophyte was estimated to be US$ 1 billion per annum to the beef cattle industry (Hoveland, 1993).   In New Zealand, the losses due to ryegrass staggers has been estimated to be worth US$ 65 million (Imlach *et al*. 2008) and in Australia the losses due to toxic *Epichloë* species causing perennial ryegrass toxicosis has been estimated to be US$ 68 million in 2012 (Webb-Ware, 2013).

Epichloë endophytes have been successfully commercialised in perennial ryegrass and tall fescue with different traits and pastoral benefits for New Zealand, Australia, South America and the USA (Johnson *et al*., 2013a; Young *et al*., 2013)**.** The identification of novel *Epichloë* endophytes has resulted in grasses being developed and bred with animal-safe properties (Easton *et al*., 2001). They can enhance the plants' survival through protection from abiotic and biotic stresses and can therefore be utilized in agriculture. Presently, novel endophyte strains have been rapidly adopted by farmers (Caradus *et al*., 2013) and are estimated to contribute approximately US$ 130 million per annum to the New Zealand economy (Johnson *et al*., 2013a).

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4.2. Commercialization of Class 2 endophytes conferring habitat-adapted fitness benefits

Based on the observation that selected Class 2 endophytes are able to confer disease, salt and heat tolerance to grasses (Rodriguez *et al*. 2008) and the subsequent finding that these tolerances can be transferred to agricultural plants (Rodriguez *et al*., 2008; Redman *et al*., 2011), these endophytes have been commercialized to improve agriculture in relation to climate changes, which may give rise to water, drought, and salination stresses (http://www.adaptivesymbiotictechnologies.com/executive-team.html). The products include *BioEnsure®-Corn* and *BioEnsure®-Rice*. *BioEnsure®-Corn* is promoted to have a 25-80% yield increase under heavy drought stress and even a 7% yield increase under low drought stress. These plants also use 25-50% less water under normal conditions as well as under low drought stress. *BioEnsure®-Rice* is promoted to cause a yield increase under drought and salt stresses as well as a 25-40% decreased water use. The products are sold as liquid formulations that are sprayed onto seeds by commercial seed treatment companies. The fungi remain dormant on the seed until germination when they establish a symbiotic association with seedlings (http://www.adaptivesymbiotictechnologies.com/press--publications.html).

4.3. Commercialization of secondary metabolites, including volatiles

Presently, a number of products based on secondary metabolites of endophytic fungi are on the market or close to market. The use of cryptocandin (Walsh, 1992) and its related compounds has been effective against a number of fungi causing diseases of skin and nails (Strobel & Daisy, 2003)   Marrone Bioinnovations has licensed *M. albus* and EPA approval for the release of this organism for use in agriculture is expected soon. It literally sterilizes the soil in which it has been placed. It is a potential replacement for methyl bromide (Gary Strobel, *personal communication*) which is now restricted from use in many countries. A strain is already on the market for decontamination of human wastes (Strobel *et al*., 2001; Strobel & Daisy, 2003). Potential applications of the volatiles produced by the new *M. albus* strains were suggested for treatment of seeds, fruits and cut flowers, as well as for soil (Ezra *et al*., 2004**).**

As discussed in section 3.4., the endophytic fungus *Daldinia concentrica* produces a mixture of volatile organic compounds which are very promising for post harvest control of dried fruits (see Fig. 4) and other plant products. Effective and safe mixtures can be assembled from the volatiles produced by *M. crispans*, and these mixtures could have commercial potential (Mitchell *et al*., 2010). In principle, the products can be used for food, flavoring and preservative purposes because each volatile component is on the FDA GRAS list. Presently, a major company is examining the volatile mixture for use as post harvest treatment of fruits and vegetables. In the meantime the product is being used to treat cheeses, and as a treatment of industrial surfaces (Gary Strobel, *personal communication*).

5. How can fungal endophytes become lost and/or their plant-beneficial activities reduced in domesticated plants?

We postulate that some wild plants have fungal endophytes which are lacking in their domesticated relatives. Under conditions of variable environmental and physiological conditions there will be selective pressure to maintain the endophyte within the plant and to keep its beneficial activity for the plant in optimal form. It seems reasonable to assume that plants can lose endophytes when they do not benefit from their presence, and also when pesticides and fungicides are frequently used. Also, in cases of low or no selective pressure, an endophyte may be less effective due to either the loss of secondary metabolites and/or because it will collect mutations against for which there is no selection. The result is a reduction in the efficacy of the endophyte and loss of beneficial activity.

The following experimental observations support these notions.
(i) Weese *et al*. (2015) collected *Rhizobium* strains from two fields on which clover was grown. One field had been nitrogen-fertilized for 22 years whereas the control field had not received nitrogen fertilizer. They compared the nitrogen fixing activity of collected *Rhizobium* strains and found that the rhizobial soil community from the nitrogen-fertilized field was less mutualistic: the inoculated clover had 17-30% less biomass and a reduced chlorophyll content.
(ii) Redman *et al*. (2011) showed that inoculation of rice plants sensitive to salt and drought with selected fungal endophytes resulted in plants which had become tolerant to these stresses. It appeared that, when the plants were grown in the absence of these stresses, endophyte colonization decreased from 100% at planting to 65%. In contrast, plants grown under continual stress maintained 100% colonization levels.
(iii) Chen et al. (2015) concluded that crop domestication can profoundly alter interactions among plants, herbivores, and their natural enemies. Domestication consistently has reduced chemical resistance against herbivorous insects, improving herbivore and natural enemy performance on crop plants. They coined the term "domestication syndrome" which they defined as the "suite of traits that commonly differ between wild and domesticated plants".
(iv) Finally, Li *et al*. (1998) proposed that the lack of host stimulus in culture media may explain why the production of biomolecules by a nascent endophyte isolate is often severely attenuated through sub-culturing.

Apparently, during domestication, both plant and endophyte can lose activities required for an optimal partnership. We hypothesize that the reasons for a loss or reduced effectiveness of endophyte(s) via the loss of certain secondary metabolites for example, can include the following:
(i) Growing plants with little or no selective pressure.
(ii) Use of systemic fungicides during *in vitro* propagation in the plant breeding process: whereas fungicides are used to prevent fungal infection from the environment, the fungal endophytes within the plant material may also be deleteriously affected.

(iii) Due to lack of selective pressure during the plant breeding process, activities such as endophyte-mediated nutrient acquisition and pathogen resistance may have been lost.

(iv) Seeds can be "cleaned" of pathogens present on and in the seeds by heat treatment. It seems likely that during this process some endophytes will also be killed.

(v) Commercial seeds are likely to contain fungicides in their coating. The fungicides may kill endophytes during storage or during subsequent germination.

(vi) The treatment of growing plants with systemic fungicides may kill all or part of the fungal endophyte population.

6. How can the loss of fungal endophytes be further prevented and/or restored?

The solution to incorporating useful fungal endophytes in crop plants lies in being aware of how fungal endophytes might be lost and then mitigating against this loss through all stages of crop breeding and management. Those managing plant germplasm resources need to consider the following:

(i) The lost part of the beneficial microbiome will be present in and on wild plants collected from the centers of origin of crop plants, the geographical area where a group of organisms was first domesticated and where they are native (Chen *et al.*, 2015). The key is to conserve not only the plants but also the whole microbiome associated with these plants. Presently, the main focus of such studies is on bacteria, especially those from the rhizosphere (Wissuwa *et al.*, 2009; Perez-Jaramillo *et al.* 2015; Bulgarelli *et al.*, 2015), but it is crucial that the focus is extended to fungi present in and on all parts of the plant.

(ii) Seeds of plants from centers of origin of crop plants have often been collected by seed companies or germplasm centres. However, they generally only make seeds available to scientists after they have been propagated to increase the amount of seed available for evaluation. However, the risk associated with propagation in the absence of the required selective pressures is that endophytes may be lost. An additional concern is that when seed is sent across national borders biosecurity regulations often require a heat or fungicidal treatment to stop the importation of pathogens. In addition to killing fungal pathogens that might be present, these treatments may also remove beneficial fungal endophytes.

(iii) Manage the endophyte with the same level of importance that we manage the host crop plant. Despite the fact that the plant may naturally select its beneficial microbiome, it can only do this if the beneficial microbes are present for selection. Therefore, it may make sense to supply the beneficial endophyte by artificial inoculation, seed treatment or other means to the plant. In dealing with fungi that have an obligate endophytic lifestyle it must be assumed that genes from both the host plants and the microbe are involved in establishing an optimal partnership. It is therefore crucial to select, identify, and characterize these genes from both partners before they are lost by lack of selective pressure.

7. Recommendations for germplasm centres, plant breeders, seed companies, microbiologists, politicians, opinion makers and the public

Primarily our recommendations have been targeted towards fungal endophytes, but in many instances the same will apply for bacterial endophytes.

To **germplasm centres** we request that recognition is given to the likelihood that seed may contain numerous fungal endophytes that may have scientific and economic importance. Storing seed at low temperature and low humidity will not only benefit the longevity of the seed itself but also the viability of any fungal endophytes they may contain. In addition ensure that high temperature and fungicidal treatment of seed is avoided.

To **plant breeders** we request that they realize that their crop of importance is often benefited by fungal endophytes and suggest they (i) check whether known beneficial microbes are lost during their breeding procedures, (ii) adapt their protocols in order to include the microbiomes, (iii) collect and sequence DNA from seeds of plants from the centers of origin before they are being propagated to identify the presence of potentially beneficial endophytes, and (iv) check whether propagation and treatment of seeds from centers of origin results in the loss of beneficial microbes, and loss or mutation of genes.

To **seed companies** we suggest they (i) check whether heat treatment and the use of synthetic as well as biological fungicides in seed coatings kills or weakens beneficial microbes within or on the seeds, (ii) check whether substances present in seed coatings kill beneficial microbes during storage and during germination, (iii) and if possible store seed containing beneficial and economically important fungal endophytes at low temperature and low humidity.

To **plant microbiologists** we suggest they (i) preserve and sequence all microbiomes of plants from the centers of origin, (ii) identify from these collections microbes beneficial in an agricultural and horticultural context, and (iii) search for novel microbial beneficial secondary metabolites, especially from the centers of origin and from tropical rain forests.

To **politicians, opinion makers and the public** we request that they have an increased awareness of the importance of (i) plant microbiomes for plant health and food production, and (ii) the preservation of wild plants and their microbiomes in their habitats. Annually, the loss of natural habitats through clearing, harvesting, fire, agricultural development, mining, or other human-oriented activities results in the inevitable loss of not only plant species of importance but also of fungal endophytes. Since these microbes may hold the key to future opportunities for a sustainable agricultural we must focus on capturing the benefits of these beneficial microbes with some urgency.

Acknowledgements

References

Aiken GE, Kirch BH, Strickland JR *et al.* Hemodynamic responses of the caudal artery to toxic tall fescue in beef heifers. *J Anim Sci* 2007;**85**:2337–45.

Aly AH, Debbab A, Proksch P. Fungal endophytes: Unique plant inhabitants with great promises. *Appl Microbiol Biotechnol* 2011;**90**:1829–45.

Andrade-Linares DR, Grosch R, Restrepo S *et al.* Effects of dark septate endophytes on tomato plant performance. *Mycorrhiza* 2011;**21**:413–22.

Ansari, M.W., Trivedi, D.K., Sahoo, R.K., Gill, S.S. and Tuteja, N. A critical review on fungi mediated plant responses with special emphasis to *Piriformospora indica* on improved production and protection of crops. *Plant Physiology and Biochemistry* 2013;**70**:403-410.

Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D. and Kursar, T.A. Are tropical fungal endophytes hyperdiverse? *Ecology Letters* 2000; **3**:267-274.

Arnold AE. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biol Rev* 2007;**21**:51–66.

Arnold A. Hidden within our botanical richness, a treasure trove of fungal endophytes. *Plant Press* 2008; November:13–5.

Bacon CW. Grass-Endophyte Research, 1977-Present, an Ecological Miasma Clarified through History. *In*: Hume DE, Latch GCM, Easton HS (eds.). *Proc. 2nd Internat. Symposium on Acremonium/Grass Interactions*. Research, Grasslands Research Centre, Palmerston North, NZ 1993;pp. 3–10.

Bacon CW. Toxic endophyte-infected tall fescue and range grasses: historic perspectives. *J Anim Sci* 1995;**73**:861–70.

Bamford PC, Norris GLF, Ward G. Flavipin production by *Epicoccum* spp. *Trans Br Mycol Soc* 1961;**44**:354–6.

Baute MA, Deffieux G, Baute R *et al.* New antibiotics from the fungus Epicoccum nigrum. I. Fermentation, isolation and antibacterial properties. *J Antibiot (Tokyo)* 1978;**31**:1099–101.

Behie SW, Zelisko PM, Bidochka MJ. Endophytic Insect-Parasitic Fungi Translocate Nitrogen Directly from Insects to Plants. *Science* 2012;**336**:1576–7.

Bertrand S, Bohni N, Schnee S *et al.* Metabolite induction via microorganism co-culture: a potential way to enhance chemical diversity for drug discovery. *Biotechnol Adv* 2014;**32**:1180–204.

Bills G, Dombrowski A, Peleaz F *et al.* Recent and future discoveries of pharmacologically acitive metabolites from tropical fungi. *In*: Watling R, Frankland JC, Ainsworth AM, *et al*. (eds.). *Tropical Mycology: Micromycetes*. 2002;**2**: 165–94. Wallingford, Oxon: CABI Publishing.

Blankenship JD, Spiering MJ, Wilkinson HH *et al.* Production of loline alkaloids by the grass endophyte, *Neotyphodium uncinatum*, in defined media. *Phytochemistry* 2001;**58**:395–401.

Bledsoe C, Klein P, Bliss LC. A survey of mycorrhizal plants on Truelove Lowland, Devon Island, N.W.T., Canada. *Can J Bot* 1990;**68**:1848–56.

Boberg JB, Ihrmark K, Lindahl BD. Decomposing capacity of fungi commonly detected in Pinus sylvestris needle litter. *Fungal Ecol* 2011;**4**:110–4.

Bouton JN, Hill , Hoveland M, McCann F *et al*. Performance of tall fescue cultivars infected with non-toxic endophytes. In *Proc. 4th Intern. Neotyphodium/Grass Interactions Symposium*. 2000; pp. 179-85. Paul VH, Dapprich PD (eds). Soest, Germany, 27-29 September 2000.

Brakhage AA. Regulation of fungal secondary metabolism. *Nat Rev Microbiol* 2013;**11**:21–32.

Brem D, Leuchtmann A. High prevalence of horizontal transmission of the fungal endophyte *Epichloë sylvatica*. *Bulletin of the Geobotanical Institute ETH* 1999;**65**:3-12.

Brown AE, Finlay R, Ward JS. Antifungal compounds produced by *Epicoccum purpurascens* against soil-borne plant pathogenic fungi. *Soil Biol Biochem* 1987;**19**:657–64.

Bulgarelli D, Garrido-Oter R, Muench PC *et al.* Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* 2015;**17**:392–403.

Caradus J, Lovatt S, Belgrave B. Adoption of forage technologies by New Zealand farmers–case studies. In *Proceedings of the International Grasslands Congress* 2013; 22:1843-5. Sydney.

Card SD, Hume DE, Davood R *et al*. Beneficial endophytic microorganisms of Brassica – a review. *Biological Control* 2015;**90:**102-12.

Card SD, Johnson LJ, Teasdale S *et al*. Deciphering microbial behaviour – the link between endophyte biology and efficacious biological control agents. *FEMS Microbiology Ecology,* 2016; doi: 10.1093/femsec/fiw114.

Chen YH, Gols R, Benrey B. Crop domestication and naturally selected species trophic interactions. *Annu Rev Entomol* 2015;**60**:35–58.

Chung K-R, Schardl CL. Sexual cycle and horizontal transmission of the grass symbiont, *Epichloë typhina*. *Mycological Research*1997;**101**:295-301.

Cunningham IJ, Hartley WJ. Ryegrass staggers. *N Z Vet J* 1959;**7**:1–7.

Daisy BH, Strobel GA, Castillo U *et al.* Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. *Microbiology* 2002;**148**:3737–41.

Demain AL. Microbial natural products: a past with a future. In: Wrigley SK, Hayes MA, Thomas R, et al. (eds.). *Biodiversity: New Leads for Pharmaceutical and Agrochemical Industries*. Cambridge, UK. The Royal Society of Chemistry, 2000; 3–16.

Dreyfuss MM, Chapela IH. Potential of fungi in the discovery of novel, low-molecular weight pharmaceuticals. *Biotechnology* 1994;**26**:49–80.

Easton HS, Christensen MJ, Eerens JPJ *et al*. Ryegrass endophyte: a New Zealand Grassland success story. *Proceedings of the New Zealand Grassland Association* 2001;**63**:37-46.

Egamberdieva D, Kamilova F, Validov S *et al.* High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. *Environ Microbiol* 2008;**10**:1–9.

Egamberdieva D, Lugtenberg B. Use of Plant Growth-Promoting Rhizobacteria to Alleviate Salinity Stress in Plants. In: *Use of Microbes for the Alleviation of Soil Stresses,* Vol 1. Miransari M (ed.). New York, NY: Springer New York, 2014;73–96.

Ernst, M., Mendgen, K.W. and Wirsel, S.G.R. (2003). Endophytic Fungal Mutualists: Seed-Borne Stagonospora Spp. Enhance Reed Biomass Production in Axenic Microcosms. *Molecular Plant-Microbe Interactions* 2003;**16**:580-587.

Ezra D, Hess WM, Strobel GA. New endophytic isolates of *Muscodor albus*, a volatile-antiobiotic-producing fungus. *Microbiology* 2004;**150**:4023–31.

Fakhro A, Andrade-Linares DR, von Bargen S *et al.* Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens. *Mycorrhiza* 2010;**20**:191–200.

Fávaro LC de L, Sebastianes FL de S, Araújo WL. *Epicoccum nigrum* P16, a Sugarcane Endophyte, Produces Antifungal Compounds and Induces Root Growth. Liles MR (ed.). *PLoS One* 2012;**7**:e36826.

Finch SC, Fletcher LR, Babu JV. The evaluation of endophyte toxin residues in sheep fat. *New Zealand Veterinary Journal*;2012; **60:** 56-60.

Findlay JA, Buthelezi S, Li G *et al.* Insect Toxins from an Endophytic Fungus from Wintergreen. *J Nat Prod* 1997;**60**:1214–5.

Fletcher L R. Grazing ryegrass/endophyte associations and their effect on animal health and performance. In: *Proceedings 2nd International Symposium on Acremonium/grass interactions: Plenary papers*. Hume DE , Latch GCM, Easton HS (Eds).1993;pp. 115-20. Palmerston North: Agresearch.

Fletcher LR, Sutherland BL, Fletcher CG. The impact of endophyte on the health and productivity of sheep grazing ryegrass-based pastures. *Ryegrass endophyte: an essential New Zealand symbiosis: Grassland Research and Practice Series* 1999;**7**:11–7.

Franken P. The plant strengthening root endophyte *Piriformospora indica*: Potential application and the biology behind. *Appl Microbiol Biotechnol* 2012;**96**:1455–64.

Friesen ML. Microbially Mediated Plant Functional Traits. *Molecular Microbial Ecology of the Rhizosphere*. 2013, **1:**87–102. Hoboken, NJ, USA: John Wiley & Sons, Inc.

Fuqua WC, Winans SC, Greenberg EP. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol* 1994;**176**:269–75.

Gallagher RT, White EP, Mortimer PH. Ryegrass Staggers: Isolation of Potent Neurotoxins Lolitrem a and Lolitrem B From Staggers-Producing Pastures. *N Z Vet J* 1981;**29**:189–90.

Gallagher RT, Smith GS, di Menna ME *et al.* Some Observations on Neurotoxin Production in Perennial Ryegrass. *N Z Vet J* 1982;**30**:203–4.

Gallagher, R.T., Hawkes, A.D., Steyn, P.S. and Vleggaar, R. Tremorgenic neurotoxins from perennial ryegrass causing ryegrass staggers disorder of livestock: Structure elucidation of lolitrem B. *Journal of the Chemical Society, Chemical Communications* 1984:614-616.

Ganley, R.J., Brunsfeld, S.J. and Newcombe, G. (2004). A community of unknown, endophytic fungi in western white pine. *Proceedings of the National Academy of Sciences of the United States of America* 2004;**101**:10107-10112.

Gao, K. and Mendgen, K. Seed-transmitted beneficial endophytic Stagonospora sp. can penetrate the walls of the root epidermis, but does not proliferate in the cortex, of Phragmites australis. *Canadian Journal of Botany* 2006;**84**:981-988.

Gill, S.S., Gill, R., Trivedi, D.K., Anjum, N.A., Sharma, K.K., Ansari, M.W., Ansari, A.A., Johri, A.K., Prasad, R., Pereira, E., Varma, A. and Tuteja, N. *Piriformospora indica*: Potential and Significance in Plant Stress Tolerance. *Frontiers in Microbiology* 2016; **7**:1-20.

Gunatilaka AAL. Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity, and implications of their occurrence. *J Nat Prod* 2006;**69**:509–26.

Haas D, Défago G. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* 2005;**3**:307–19.

Hallmann J, Quadt-Hallmann A, Mahaffee WF *et al.* Bacterial endophytes in agricultural crops. *Can J Microbiol* 1997;**43**:895–914.

Hardoim PR, van Overbeek LS, Berg G *et al.* The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiol Mol Biol Rev* 2015;**79**:293–320.

Harper JK, Arif AM, Ford EJ *et al.* Pestacin: a 1,3-dihydro isobenzofuran from *Pestalotiopsis microspora* possessing antioxidant and antimycotic activities. *Tetrahedron* 2003;**59**:2471–6.

Hopkins AA, Alison MW. Stand Persistence and Animal Performance for Tall Fescue Endophyte Combinations in the South Central USA. *Agronomy Journal* 2006; **98**: 1221-6

Horn WS, Simmonds MSJ, Schwartz RE *et al.* Phomopsichalasin, a novel antimicrobial agent from an endophytic *Phomopsis* sp. *Tetrahedron* 1995;**51**:3969–78.

Hoveland CS. Importance and economic significance of the *Acremonium* endophytes to performance of animals and grass plant. *Agric Ecosyst Environ* 1993;**44**:3–12.

Hunter DM, Milner RJ, Spurgin PA. Aerial treatment of the Australian plague locust, Chortoicetes terminifera (Orthoptera: Acrididae) with *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). *Bull Entomol Res* 2001;**91**:93–9.

Hussain H, Root N, Jabeen F *et al.* Seimatoric acid and colletonoic acid: Two new compounds from the endophytic fungi, *Seimatosporium* sp. and *Colletotrichum* sp. *Chinese Chem Lett* 2014;**25**:1577–9.

Hussain H, Al-Harrasi A, Krohn K *et al.* Phytochemical investigation and antimicrobial activity of *Derris scandens*. *J King Saud Univ - Sci* 2015;**27**:375–8.

Hyde, K.D. and Soytong, K. (2008). The fungal endophyte dilemma. *Fungal Diversity*, **33**, 163-73.

Ikawa M, McGratten CJ, Burge WR *et al.* Epirodin, a polyene antibiotic from the mold *Epipoccum nigrum*. *J Antibiot (Tokyo)* 1978;**31**:159–61.

Imlach WL, Finch SC, Dunlop J *et al*. The Molecular Mechanism of "Ryegrass Staggers", a Neurological Disorder of K^+ Channels. *Journal of Pharmacology and Experimental Therapeutics* 2008;**327**:657-64.

Johnson, J.M., Alex, T. and Oelmüller, R. *Piriformospora indica*: The versatile and multifunctional root endophytic fungus for enhanced yield and tolerance to biotic and abiotic stress in crop plants. *Journal of Tropical Agriculture* 2014;**52**:103-122.

Johnson LJ, De Bonth ACM, Briggs LR *et al.* The exploitation of epichloae endophytes for agricultural benefit. *Fungal Divers* 2013a;**60**:171–88.

Johnson LJ, Koulman A, Christensen M. *et al.* An extracellular siderophore is required to maintain the mutualistic interaction of *Epichloë festucae* with *Lolium Perenne PLOS Pathogens*. 2013b;**9**: e1003332.

Johnson, J.M., Alex, T. and Oelmüller, R. (2014). *Piriformospora indica*: The versatile and multifunctional root endophytic fungus for enhanced yield and tolerance to biotic and abiotic stress in crop plants. Journal of Tropical Agriculture, 52, 103-122.

Kemami Wangun H V, Hertweck C. Epicoccarines A, B and epipyridone: tetramic acids and pyridone alkaloids from an *Epicoccum* sp. associated with the tree fungus *Pholiota squarrosa*. *Org Biomol Chem* 2007;**5**:1702–5.

Kivlin, S.N., Emery, S.M. and Rudgers, J.A. (2013). Fungal symbionts alter plant responses to global change. *American Journal of Botany*, **100**, 1445-1457.

Kumar, M., Yadav, V., Tuteja, N. and Johri, A.K. (2009). Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. *Microbiology*, 155, 780-790.

Latch GCM, Christensen MJ. Ryegrass endophyte, incidence, and control. *New Zeal J Agric Res* 1982;**25**:443–8.

Latch GCM, Christensen MJ. Artifical infection of grasses with endophytes. *Ann aook Biol 1985; **107**; 17-24.*

Leuchtmann A, Bacon CW, Schardl CL *et al.* Nomenclatural realignment of *Neotyphodium* species with genus Epicholë. *Mycologia* 2014;**106**:202–15.

Li JY, Harper JK, Grant DM *et al.* Ambuic acid, a highly functionalized cyclohexenone with antifungal activity from *Pestalotiopsis* spp. and *Monochaetia* sp. *Phytochemistry* 2001;**56**:463–8.

Li JY, Sidhu RS, Ford EJ *et al.* The induction of taxol production in the endophytic fungus--*Periconia* sp from Torreya grandifolia. *J Ind Microbiol Biotechnol* 1998;**20**:259–64.

Li JY, Strobel G, Harper J *et al.* Cryptocin, a potent tetramic acid antimycotic from the endophytic fungus *Cryptosporiopsis* cf. *quercina*. *Org Lett* 2000;**2**:767–70.

Li JY, Strobel GA. Jesterone and hydroxy-jesterone antioomycete cyclohexenone epoxides from the endophytic fungus *Pestalotiopsis jesteri*. *Phytochemistry* 2001;**57**:261–5.

Lomer CJ, Bateman RP, Johnson JL *et al*. Biological Control of Locusts and Grasshopers. *Annu Rev Entomol*. 2001:667–702.

Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol* 2009;**63**:541–56.

Madrigal C, Tadeo JL, Melgarejo P. Relationship between flavipin production by *Epicoccum nigrum* and antagonism against *Monilinia laxa*. *Mycol Res* 1991;**95**:1375–81.

Madrigal C, Melgarejo P. Morphological effects of *Epiccocum nigrum* and its antibiotic flavipin on *Monilinia laxa*. *Can J Bot* 1995;**73**:425–31.

Márquez LM, Redman RS, Rodriguez RJ *et al.* A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. *Science* 2007;**315**:513–5.

Mejía LC, Rojas EI, Maynard Z *et al.* Endophytic fungi as biocontrol agents of *Theobroma* cacao pathogens. *Biol Control* 2008;**46**:4–14.

Mercado-Blanco J. Life of microbes inside the plant. In: *Principles of Plant-Microbe Interactions- Microbes for Sustainable Agriculture*; Lugtenberg B (Ed) 2015; pp. 25-32. Springer.

Mitchell AM, Strobel GA, Moore E *et al.* Volatile antimicrobials from *Muscodor crispans*, a novel endophytic fungus. *Microbiology* 2010;**156**:270–7.

Murphy BR, Doohan FM, Hodkinson TR. Yield increase induced by the fungal root endophyte *Piriformospora indica* in barley grown at low temperature is nutrient limited. *Symbiosis* 2014;**62**:29–39.

Murphy BR, Doohan FM, Hodkinson TR. Fungal root endophytes of a wild barley species increase yield in a nutrient-stressed barley cultivar. *Symbiosis* 2015a:1–7.

Murphy BR, Doohan FM, Hodkinson TR. Persistent fungal root endophytes isolated from a wild barley species suppress seed-borne infections in a barley cultivar. *BioControl* 2015b;**60**:281–92.

Netzker T, Fischer J, Weber J *et al.* Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. *Front Microbiol* 2015;**6**:1–13.

Newsham KK. A meta-analysis of plant responses to dark septate root endophytes. *New Phytol* 2011;**190**:783–93.

Oberwinkler, F., Riess, K., Bauer, R., Selosse, M.A., Weiß, M., Garnica, S. and Zuccaro, A. Enigmatic Sebacinales. *Mycological Progress* 2013;**12**:1-27.

Oelmüller, R., Sherameti, I., Tripathi, S. and Varma, A. *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. *Symbiosis* 2009;**49**:1-17.

Ola ARB, Thomy D, Lai D *et al.* Inducing secondary metabolite production by the endophytic fungus *Fusarium tricinctum* through coculture with Bacillus subtilis. *J Nat Prod* 2013;**76**:2094–9.

Oliver JW. Pathophysiologic Response to Endophyte Toxins. *Neotyphodium in Cool-Season Grasses*. Oxford, UK: Blackwell Publishing Ltd, 2005; 291–304.

Pennell CGL, Popay AJ, Ball OJP *et al.* Occurrence and impact of pasture mealybug (Balanococcus poae) and root aphid (Aploneura lentisci) on ryegrass (Lolium spp.) with and without infection by *Neotyphodium* fungal endophytes. *New Zeal J Agric Res* 2005;**48**:329–37.

Perez‑Jaramillo JE, Mendes R, Raaijmakers JM. Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Mol Biol*,2015; DOI 10.1007/s11103-015-0337-7

Philipson MN, Christey MC. The relationship of host and endophyte during flowering, seed formation, and germination of Lolium perenne. *New Zeal J Bot* 1986;**24**:125–34.

Pliego C, Kamilova F, Lugtenberg B. Plant Growth-Promoting Bacteria: Fundamentals and Exploitation. In: *Bacteria in Agrobiology: Crop Ecosystems*. Maheshwari DK (ed.). Springer Berlin Heidelberg; 2011:295–343.

Popay AJ, Prestidge RA, Rowan DD *et al.* The role of *Acremonium lolii* mycotoxins in insect resistance of perennial ryegrass (Lolium perenne). In: *Proceedings of the International Symposium on Acremonium /Grass Interaction*. Quisenberry SS, Joost R. (eds). Baton Rouge, 1990, 44–8.

Porras-Alfaro A, Bayman P. Hidden Fungi, Emergent Properties: Endophytes and Microbiomes. *Annu Rev Phytopathol* 2011;**49**:291–315.

Porter, J.K. Chemical constituents of grass endophytes. In: *Biotechnology of Endophytic Fungi of Grasses* 1994. Bacon, C.W., White Jr., J.F. (Eds), CRC, Boca Raton, FL, pp. 103–23.

Prestidge R, Pottinger RP, Barker GM. An association of Lolium endophyte with ryegrass resistance to Argentine stem weevil. In: *35th New Zealand weed and pest control conference* 1982, pp 199–222.

Prestidge RA, Gallagher RT. Lolitrem B - a stem weevil toxin isolated from Acremonium-infected ryegrass. In: *38th New Zealand weed and pest control conference* 1985, pp 38–40

Qiang, X., Weiss, M., Kogel, K.H. and Schäfer, P. *Piriformospora indica*-a mutualistic basidiomycete with an exceptionally large plant host range. *Molecular Plant Pathology* 2012;**13**:508-518.

Quesada Moraga E, Herrero N, Zabalgogeazcoa I. 2014. Entomopathogenic and nematophagous fungal endophytes. Chapter 19 in: Advances in Endophytic Research, VC Verma, AC Gange, Eds. Springer-Verlag (Pvt.) Ltd, Heidelberg, Germany pp.85-99.

Rabiey M, Ullah I, Shaw MW. The endophytic fungus *Piriformospora indica* protects wheat from fusarium crown rot disease in simulated UK autumn conditions. *Plant Pathology* 2015; Doi: 10.1111/ppa.12335

Redman RS, Sheehan KB, Stout RG *et al.* Thermotolerance generated by plant/fungal symbiosis. *Science* 2002;**298**:1581.

Redman RS, Kim YO, Woodward CJDA *et al.* Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: A strategy for mitigating impacts of climate change. *PLoS One* 2011;**6**, DOI: 10.1371/journal.pone.0014823.

Reed KFM, Lean IJ. *Perennial Ryegrass Toxicosis in Australia: Proceedings of a Symposium Held on Friday 18 March 2005 at Attwood, Victoria*. Meat and Livestock Australia, 2005.

Rodriguez Estrada, A.E., Jonkers, W., Corby Kistler, H. and May, G. (2012). Interactions between *Fusarium verticillioides*, *Ustilago maydis*, and *Zea mays*: An endophyte, a pathogen, and their shared plant host. *Fungal Genetics and Biology*, **49**, 578-587.

Rodriguez RJ, Henson J, Van Volkenburgh E *et al.* Stress tolerance in plants via habitat-adapted symbiosis. *ISME J* 2008;**2**:404–16.

Rodriguez RJ, White JF, Arnold AE *et al.* Fungal endophytes: diversity and functional roles. *New Phytol* 2009;**182**:314–30.

Rowan DD, Dymock JJ, Brimble MA. Effect of fungal metabolite peramine and analogs on feeding and development of Argentine stem weevil (Listronotus bonariensis). *J Chem Ecol* 1990;**16**:1683–95.

29

Rowan DD. Lolitrems, peramine and paxilline: Mycotoxins of the ryegrass/endophyte interaction. *Agric Ecosyst Environ* 1993;**44**:103–22.

Saikkonen K, Faeth SH, Helander M *et al.* Fungal endophytes: a Continuum of Interactions with Host Plants. *Annu Rev Ecol Syst* 1998;**29**:319–43.

Schardl CL, Leuchtmann A, Spiering MJ. Symbioses of grasses with seedborne fungal endophytes. *Annu Rev Plant Biol* 2004;**55**:315–40.

Schardl CL, Panaccione DG. Biosynthesis of Ergot and Loline Alkaloids. In: *Neotyphodium in Cool-Season Grasses*. Roberts CA, West CP, Spiers DE (eds). Oxford, UK: Blackwell Publishing Ltd, 2005;73–92.

Schroeckh V, Scherlach K, Nützmann H-W *et al.* Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proc Natl Acad Sci U S A* 2009;**106**:14558–63.

Schulz B. Endophytic fungi: a source of novel biologically active secondary metabolites . *In* British Mycological Society, *International symposium proceedings: bioactive fungal metabolites—impact and exploitation*.2001; p. 20. University of Wales, Swansea, Wales.

Schulz B, Boyle C. The endophytic continuum. *Mycol Res* 2005;**109:** 661–86.

Schulz BJE. Mutualistic interactions with fungal root endophytes. In: *Microbial Root Endophytes*. Schulz BJE, Boyle CJC, Sieber TN (eds) 2006; **9:**261–79. Berlin, Heidelberg: Springer Berlin Heidelberg.

Shrivastava S. & Varma A. From *Piriformospora indica* to Rootonic: A review. *Afr. J. Microbiol. Res*. 2014; **8**:2984-2992.

Siegel MR, Bush LP. Importance of endophytes in forage grasses, a statement of problems and selection of endophytes. In: *Biotechnology of Endophytic Fungi of Grasses*. Bacon CW, White JF (eds). Boca Raton, FL: CRC Press, 1994;135–50.

Stinson M, Ezra D, Hess WM *et al.* An endophytic *Gliocladium* sp. of Eucryphia cordifolia producing selective volatile antimicrobial compounds. *Plant Sci* 2003;**165**:913–22.

Strobel GA, Miller RV, Miller C. *et al*. Cryptocandin, a potent antimycotic from the endophytic fungus *Cryptosporiopsis cf. quercina. Microbiology* 1999; **145:**1919–26.

Strobel G, Daisy B. Bioprospecting for Microbial Endophytes and Their Natural Products Bioprospecting for Microbial Endophytes and Their Natural Products. *Microbiol Mol Biol Rev* 2003;**67**:491–502.

Strobel G, Ford E, Worapong J *et al.* Isopestacin, an isobenzofuranone from *Pestalotiopsis microspora*, possessing antifungal and antioxidant activities. *Phytochemistry* 2002;**60**:179–83.

Strobel GA, Dirkse E, Sears J *et al.* Volatile antimicrobials from *Muscodor albus*, a novel endophytic fungus. *Microbiology* 2001;**147**:2943–50.

Strobel GA. Bioprospecting-fuels from fungi. *Biotechnol Lett* 2015;**37**:973–82.

Strobel SA, Strobel GA. Plant endophytes as a platform for discovery-based undergraduate science education. *Nat Chem Biol* 2007;**3**:356–9.

Takemoto D, Kamakura S, Saikia S *et al*. Polarity proteins Bem1 and Cdc24 are components of the filamentous fungal NADPH oxidase complex. *Proceedings of the National Academy of Sciences, USA*, 2011;**108**, 2861-6.

Takemoto D, Tanaka A, Scott B. A p67Phox-like regulator is recruited to control hyphal branching in a fungal-grass mutualistic symbiosis. *Plant Cell*, 2006;**18:** 2807-21.

Tan, R.X. and Zou, W.X. Endophytes: A rich source of functional metabolites. *Natural Product Reports* 2001:**18**:448-459.

Tanaka A, Christensen MJ, Takemoto D *et al.* Reactive oxygen species play a role in regulating a fungus-perennial ryegrass mutualistic interaction. *Plant Cell* 2006;**18:**1052-66.

Tanaka A, Takemoto D, Hyon GS *et al*. NoxA activation by the small GTPase RacA is required to maintain a mutualistic symbiotic association between *Epichloe festucae* and perennial ryegrass. *Molec Microbiol* 2008;**68:**1165-78.

Tapper BA, Lane GA. Janthitrems found in a *Neotyphodium* endophyte of perennial ryegrass. In *Proceedings of the 5th International Symposium on Neotyphodium/Grass interactions.* Kallenbach R, Rosenkrans CJ, Lock TR (Eds). 2004;p. 301. Fayetteville, Arkansas, USA.

Thompson F N, Stuedemann JA, Hill NS. Anti-quality factors associated with alkaloids in eastern temperate pasture. *J. Range Management* 2001;**54**:474–89.

Unnikumar, K.R., Sree, K.S. and Varma, A. *Piriformospora indica*: A versatile root endophytic symbiont. *Symbiosis* 2013;**60**:107-113.

Van Brussel AAN, Zaat SAJ, Canter Cremers H *et al.* Role of plant root exudate and Sym plasmid-localized nodulation genes in the synthesis by *Rhizobium leguminosarum* of Tsr factor, which causes thick and short roots on common vetch. *J Bacteriol* 1986;**165**:517–22.

Varma A, Bajaj R, Agarwal A, Asthana A & Rajpal K. Memoirs of Rootonic- the magic Fungus: Promotes agriculture, *Horticulture and Forest productivity* 2013. Amity University Uttar Pradesh Press Noida, India

Varughese T, Rios N, Higginbotham S *et al.* Antifungal depsidone metabolites from *Cordyceps dipterigena*, an endophytic fungus antagonistic to the phytopathogen Gibberella fujikuroi. *Tetrahedron Lett* 2012;**53**:1624–6.

Vázquez de Aldana, B.R., Bills, G. and Zabalgogeazcoa, I. (2013). Are endophytes an important link between airborne spores and allergen exposure? *Fungal Diversity*, **60**, 33-42.

Vega FE, Simpkins A, Aime MC *et al.* Fungal endophyte diversity in coffee plants from Colombia, Hawai, Mexico and Puerto Rico. *Fungal Ecol* 2010;**3**:122–38.

Verma S, Varma A, Rexer K-H *et al. Piriformospora indica*, gen. et sp. nov., a New Root-Colonizing Fungus. *Mycologia* 1998;**90**:896.

Vidal S, Jaber LR. Entomopathogenic fungi as endophytes : plant – endophyte – herbivore interactions and prospects for use in biological control. *Curr Sci* 2015;**109**:46–54.

Walsh TA. Inhibitors of β-glucan synthesis. In: *Emerging Targets in Antibacterial and Antifungal Chemotherapy.* Sutcliffe J, Georgopapadakou NH (eds). London and New York: Champman and Hall, 1992; 349–73.

Webb-Ware, J. 2013. Ryegrass endophyte toxins. In Proceedings of Red Meat Updates: Meat and   Livestock Australia & Macquarie Franklin. P. 22. *www.macquariefranklin.com.au/_.../Red_Meat_Updates_Proceedings*

Weber RWS, Anke H. Effects of endophytes on colonization by leaf surface microbiota. *Microbial Ecology of Aerial Plant Surfaces*. Wallingford: CABI, 2006; 209–22.

Weese DJ, Heath KD, Dentinger BTM *et al.* Long-term nitrogen addition causes the evolution of less-cooperative mutualists. *Evolution (N Y)* 2015;**69**:631–42.

Weiss M, Selosse MA, Rexer KH *et al.* Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycological research* 2004;**108**:1003-10.

Wissuwa M, Mazzola M, Picard C. Novel approaches in plant breeding for rhizosphere-related traits. *Plant Soil* 2009;**321**:409–30.

Worapong J, Strobel G, Ford EJ *et al. Muscodor albus* anam. gen. et sp. nov., and endophyte from *Cinnamomum zeylanicum*. *Mycotaxon* 2001;**79**:67–79.

Wu C, Kim HK, Van Wezel GP *et al.* Metabolomics in the natural products field - A gateway to novel antibiotics. *Drug Discov Today Technol* 2015a;**13**:11–7.

Wu C, Zacchetti B, Ram AFJ *et al.* Expanding the chemical space for natural products by *Aspergillus-Streptomyces* co-cultivation and biotransformation. *Sci Rep* 2015b;**5**:10868.

Young CA, Felitti S, Shields K *et al.* A complex gene cluster for indole-diterpene biosynthesis in the grass endophyte *Neotyphodium lolii*. *Fungal Genet Biol* 2006;**43**:679–93.

Young CA, Hume DE, McCulley RL. Forages and pastures symposium: Fungal endophytes of tall fescue and perennial ryegrass: Pasture friend or foe? *Journal of Animal Science* 2013;**91**,2379-94.

Zhang HW, Song YC, Tan RX. Biology and chemistry of endophytes. *Nat Prod Rep* 2006;**23**:753–71.

Zhang Y, Liu S, Che Y *et al.* Epicoccins A-D, epipolythiodioxopiperazines from a Cordyceps-colonizing isolate of *Epicoccum nigrum*. *J Nat Prod* 2007;**70**:1522–5.

Zou WX, Meng JC, Lu H *et al.* Metabolites of *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mongolica*. *J Nat Prod* 2000;**63**:1529–30.
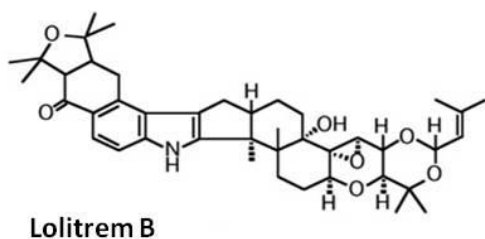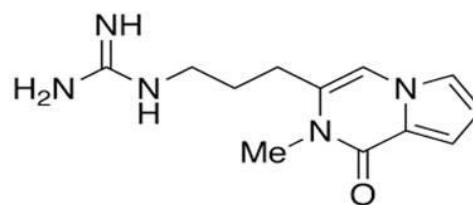
Fig. 1. Molecular explanation of the ryegrass staggers story. Sheep grazing on perennial ryegrass can contract a neuromuscular condition designated as ryegrass staggers **(A).** The condition is caused by certain strains of *Epichloë* fungal endophytes. The offending endophyte could be removed by fungicide treatment. Grazing on endophyte-free grass did not cause the disease anymore but ryegrass persistance was seriously affected by damage caused by the insect Argentine stem weevil **(B).** Subsequent reasearch has shown that the endophyte produced two major secondary metabolites: the neurotoxin **lolitrem B** which is causing ryegrass staggers and **peramine** as the major insect feeding deterrent. For details, see sections **2.2** and **3.5**. Both figures have been used freely in the public domain for many years.
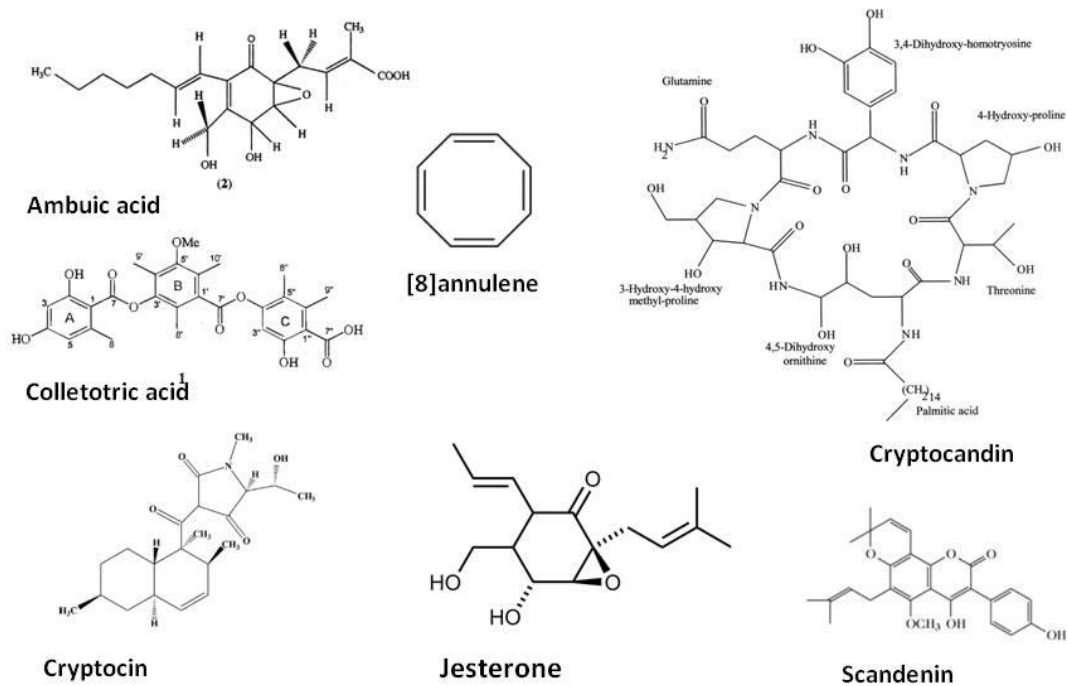
Fig. 2. Examples of some well-characterized anti-microbial secondary metabolites from endophytic fungi.

Isopestacin   Pestacin
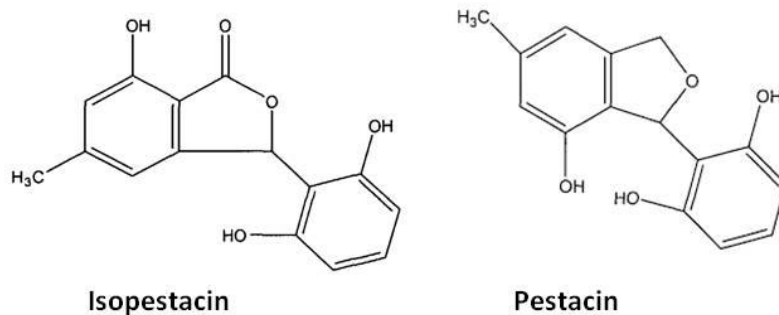
Fig. 3. Examples of anti-oxidant secondary metabolites from endophytic fungi with anti-microbial activity.
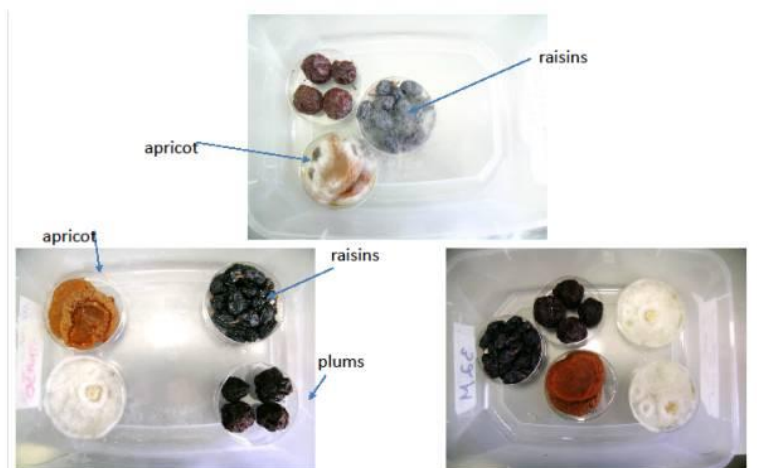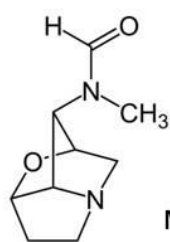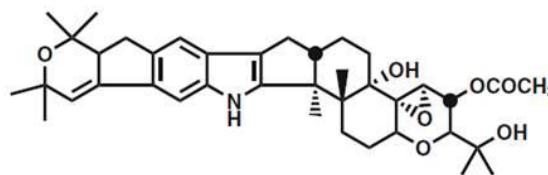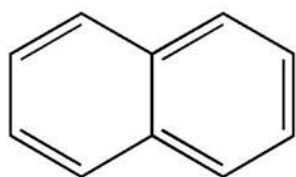
Fig. 4. Prevention from rotting of dried fruits by volatiles produced by the endophytic fungus *Daldinia concentrica*. The volatile mixture consists of at least 28 compounds. This figure has not been published previously and was kindly provided by dr David Ezra and dr Orna Liarzi.
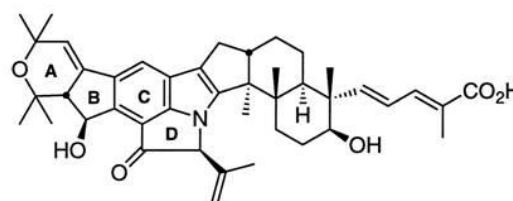
Fig. 5. Examples of some well-characterized anti-insect secondary metabolites from endophytic fungi. See **Fig. 1**. for the structure of the insect feeding deterrent peramine. The structure of epoxy-janthitrem I is from Johnson *et al*. (2013a).