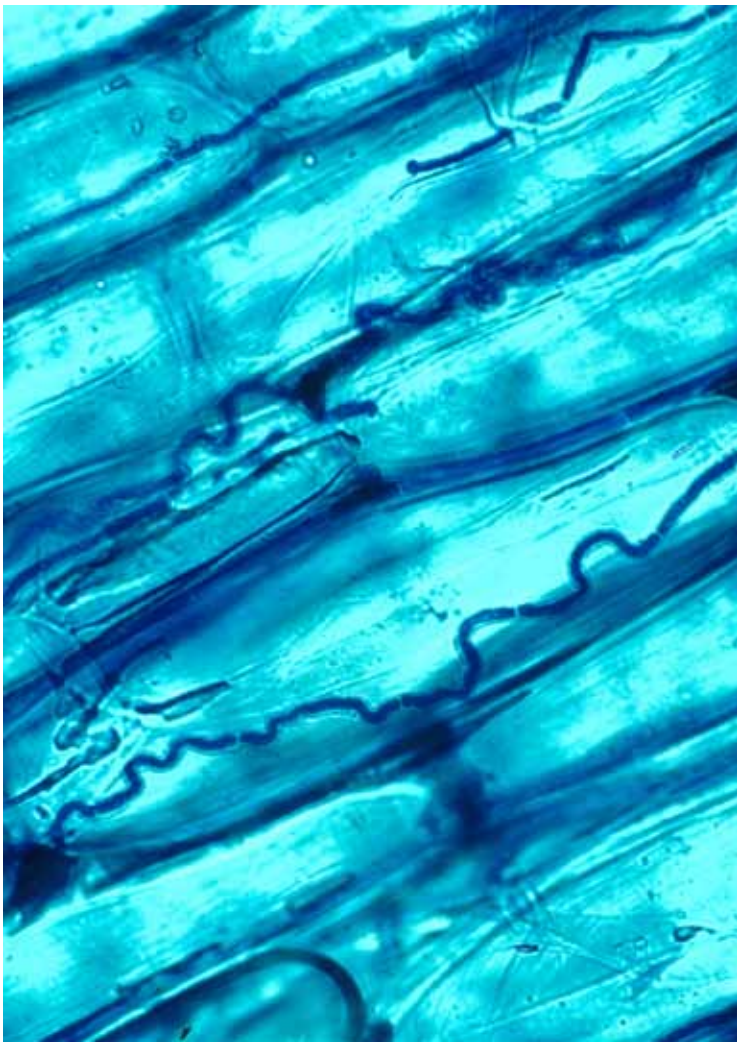
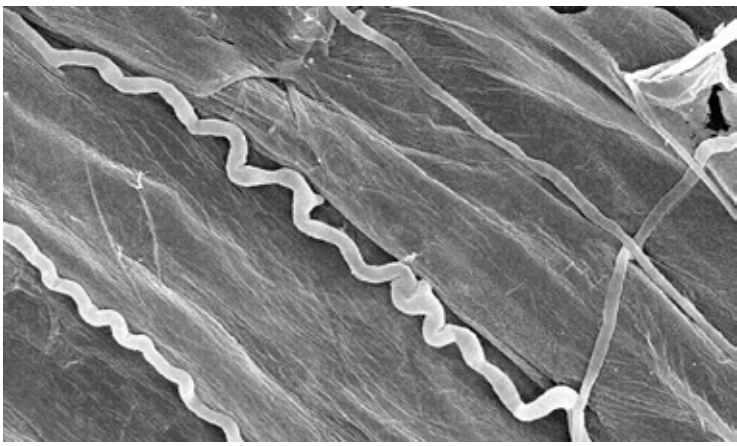
Researching Endophytes in UK Sports Turf

Jonathan Knowles from Myerscough College unveils his latest research findings on endophytes – potentially a solution for sustainable turf management





TOP: The sheath cells of tall fescue stained blue with endophytic hyphae threading intercellularly X400 magnification. Image sourced - Prof. N. Hill, University of Georgia.

ABOVE: Electron Scanning Microscope image of endophytic hyphae (tubular, white and branching) situated here between the cell walls of the sheath tissue of tall fescue. Image sourced - Prof. N. Hill, University of Georgia.

RIGHT PAGE: Image from the 2005 investigation of the suppression of red thread (Laetisaria fuciformis) and pink patch (Limnomyces roseipellis) using endophyte infected red fescues. From the two plots it is clear to see endophyte infected turf on the right suppressing the onset of disease. Image source Dr. S. Bonos, Rutgers University.

Relationships

All plants have relationships and associations with animals, invertebrates, other plants, and microbes. This relationship or symbiosis can be beneficial, antagonistic or neither. Turf diseases are a good example of an antagonistic association and conversely, mycorrhizal fungi are a classic example of a beneficial relationship. In this article I want to introduce, or at least review a symbiosis which may be new to you; a clandestine microbe - the aboveground fungal-endophyte.

Greenkeeping, or sports turf management, is a skill requiring specialist training, education and an understanding of the balance between ecology, horticulture and agriculture.

I want to discuss how microscopic fungi could potentially open up a new area of knowledge and broaden the understanding and fusion between ecology and sports turf. This area of microbe-interaction is already making progress in crop biotechnology and we are starting to see some early marketing for endophyte-infected seeds for the sports turf market already. The truth is this type of microbe-interaction has been applied and used in turf and grassland management in other countries such as New Zealand for many years.

Defining an Endophyte

An endophyte is a symbiont, typically a fungal or bacterial infection that lives within the plant. Some endophytes spend part of their life within the plant, while others spend all their lifecycle within the plant. Mycorrhizae is described as an endophyte, however, this article focuses on the fungal-endophytes that spend their life in the aerial shoots and leaves (plate 1 and 2).

Grass-Endophyte Ecology & Sports Turf

The grass-endophytes symbiosis is not new knowledge. In wild grassland ecology there is a rich source of information discussing the relationship – good and bad.

At the moment sports turf endophyte research is limited, nevertheless, many of the grasses found in ecology and agricultural research are the commonly used in sports turf. So, from what we understand at the moment, there are around six different species of fungal-endophyte infecting UK sports turf species.

That is they have the potential for infection, note we don't have the data to suggest distribution or infection levels in UK sports turf just yet. These six species belong to one family of fungal endophyte - Clavicipitaceae.

A mycologist may be familiar with ergot and choke the pathogenic fungal infections found in grasslands and pasture of rye-grass, cocks foot, tall fescue, red fescues, bent-grasses and purple moor grass.

These antagonistic endophyte infections are caused by Epichloe spp. and Claviceps purpurea. Neither of these present an obvious threat to sports turf surfaces,

Quiz

1. Select the three correct reasons why endophytes could benefit sports turf?

2. Where is the evidence that endophytes in sports turf may be beneficial?

3. There is no evidence of Poa annua being endophyte infected in UK sports turf. However, what species of endophyte could we

especially as they are diseases of the inflorescence. However, seed houses may have concerns over choke, especially as it known to castrate some turf species. Interestingly, for sports turf there is one prominent fungal-endophyte of significance - Neotyphodium spp. a derivative of Epichloe spp., for which infection is transmitted via the seed of the next generation.

Importantly, this means there are no signs of disease or the associated outward visible growing fungal parts, such as sclerotia or conidia. So this type of endophyte (Neoptyphodium) typical to our sports turf species is asymptomatic, hence it's secretive infection.

The Justification of Endophyte Research in Sports Turf

Endophytes have been subject to many grassland, forage and ecology research studies mostly associated with the aspects of grass growth and development.

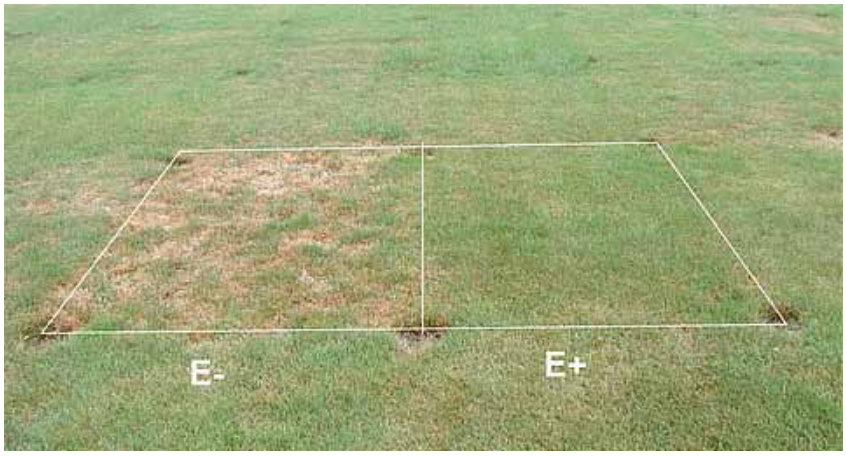
Neotyphodium infection has been reported to have an effect on increased leaf area, tillering, root development, drought tolerance, pest resistance, nutrient deficient soil, and even an increased competitive ability against inter and intra-specific species. This suggests infected grasses are invasive in areas of non-infected broadleaf weeds and grasses. However, some studies show little to no effect of the infection, and there are some contradictions.

These complications could be related to the environmental conditions, grass species, the interacting endophyte species, and even the research methodology used. When these variables are combined with the fact that the symbiotic association between endophyte and host is changeable, difficulties arise.

The association can be beneficial (mutualistic) and in contrast, under other conditions it can be detrimental (parasitic), so it can be difficult to conclude the benefits or otherwise. Indeed the benefits of the association have to be interpreted for the context in which they are set. It's not clear cut, these associations must be tested in a sports turf context to realise if the endophytes matter to the grasses and playing surfaces for sport.

Endophyte Research Matters

Stateside, Rutgers University are slightly ahead of the game studying endophytes and sports turf (plate 3). Their research has seen a Neotyphodium endophyte-mediated resistance to Dollar Spot and Red



expect to detect living within Poa annua?

4. What methods are being used to detect infection at the University of Central Lancashire and Myerscough College?

5. The immunoblot membrane image, on page 46, shows endophyte-infected grasses.
What percentage on the membrane is infected?

GLOSSARY

Endophyte Endo- meaning within and -Phyte meaning plant. Endophytes are typically fungal or bacterial. But not limited to viral or any other microbial interaction within the plant. Endophytes are obligate biotrophs.

Clavicipitaceae A family of fungi which consist of more than forty genera. Claviceps purpurea is an example, commonly known as Ergot. This grass disease leaves blackened fungal parts extruding (sclerotia) from the inflorescence.

Alkaloid A naturally forming chemical compound. Toxic alkaloids are common in fungal-endophytes. These toxins can cause Ergotisim when consumed by humans (in cereals). Many beneficial drugs such as anti-cancer, anti-viral, anti-malarial, and even against migraines (ergotamine). All are derived from endophytic alkaloids.

Ecosystem A community of living organisms within an environment. Where both biotic and abiotic elements are considered to be linked through nutrient cycles and energy flows.

Immunoblot A commonly used analysis method for the recognition of anti-bodies.

DNA Sequencing A process for determining the order of the nucleotide molecules specific to the DNA being analysed.

Neotyphodium Genera of fungal-endophytes, for which there are fourteen species. Reproduction by asexual or vertical means. Example species: N. lolii. Example host grasses: Festuca, Lolium, Bromus, Melica, and Poa.

Epichloe Genera of fungal-endophyte, for which there are ten species. Reproduction by Sexual or horizontal means. Example species: E.typhina. Example host grasses: Agrostis, Bromus, Dactylis, Elymus and Poa.

Choke See Epichloe.

Interspecific competition Competition between different species.

Intraspecific competition Competition between the same species.

Ergot See Clavicipitaceae

MycorrhizaeA mutualistic association between root and fungi. Grasses typical form associations with vesicular arbuscular mycorrhizae.

Sheath tissue The basal part of the grass. Leaves surround the stem in multiple layers.

Symbiosis An association or relationship between individual species. A symbiotic association can be antagonistic, parasitic, mutualistic, or communalistic (no benefit or determent to host).

Sustainability An approach which safeguards the future by limiting environmental damage, maintaining social equity and enduring economic demands.

Inflorescence The flower head of grasses. An aggregation of flowers on the stem.

Mutualistic A beneficial association for both host and symbiont.

Parasitic A beneficial association for symbiont organism or parasite. Causes harm, not necessarily immediate death.

Biotechnology Loosely means; applying technology that uses a biological system, living organism, or derivative whether modified or not.

Propiconazole Active ingredient of a broad-spectrum fungicide. Example, Syngenta's Banner Maxx to control Fusarium, dollar spot, anthracnose, rhizoctonia and bipolaris.

Argentine stem weevil A small brown weevil (3 mm in length). A significant turfgrass pest in New Zealand.

Claviceps purpurea See Clavicipitaceae

Thread in red fescues. Potentially we could be unearthing a biotechnical answer for the future of sustainable turf disease management. But, there is no evidence to suggest endophytes benefit UK sports turf.

While we interpret the benefits we must also understand the relationship being changeable. For example, it has been discovered that a Neotyphodium sp. can prevent wilt during drought, however, following the drought period the infection becomes parasitic diverting the plants resources for its own survival weakening the plant to further stress.

Another example is the hastening of the grass's maturity. There is some evidence to suggest endophyte-infected plants die prematurely.

Possibly not an issue with an over-seeding regime, but we can assume a weakening of the sward that could be susceptible to the threats of disease, pests and weeds.

Also there is evidence that alkaloid producing endophytes have the ability to deter pests such as the Argentine stem weevil in New Zealand, however there is no evidence the effect would be similar in UK sports turf against pests such

BELOW: Immunoblot testing carried out at Myerscough College. This membrane is imprinted with seven cultivars (numbered) with ten replicates. For clarity the insert magnifies an example of an infected reaction. On this membrane there are four positive infections. Control seeds at the bottom show infected to the left and non-infected above the red line to the right. All these cultivars are available for UK sports turf.

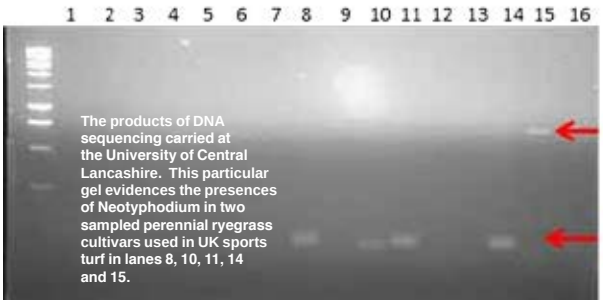
as chafer grubs or even parasitic nematodes.

After all, many of the cool-season turf grasses have evolved in northern Europe with the endophytes and the associated turf feeding invertebrates.

It is reasonable to hypothesize the pests could be unaffected by the toxins, without the testing we would not know.

Similarly for nematodes, it is only a limited species of endophyte that produces the alkaloids that have the anti-biological toxins to resist invertebrate herbivores.

There are many species of endophytes, it may be a case that certain endophyte infections hold beneficial traits against herbivorous insects while other species are ineffective.



The products of DNA sequencing carried at the University of Central Lancashire. This particular gel evidences the presences of Neotyphodium in two sampled perennial ryegrass cultivars used in UK sports turf in lanes 8, 10, 11, 14 and 15.

about the author

Jonathan Knowles

Jonathan started his greenkeeping career 20 years in Nottinghamshire. He is part of the Myerscough College team supporting the delivery of sports turf qualifications. Through a scholarship at the University of Central Lancashire and support of the college he is undertaking a PhD, researching the interaction of fungal endophytes in sports turf. For further information about the research please contact him at jknowles@uclan.ac.uk

Even so there needs to be an understanding of endophytes in our surfaces.

The understanding or management of endophytes will need introducing as a new area of knowledge in sports turf.

A good example is the storage of seed; infection in the seed will only survive for around a year stored at 21C, however it is reported to persist for a decade stored at 4C. Moreover, there is an issue with the application of commonly used broad-spectrum fungicides eradicating the infection, particularly by Propiconazole.

Endophyte infected cultivars are starting to appear in sports turf management and quite possibly managers are starting in earnest to incorporate these endophytes into the surface with good intentions.

However, they could be unwittingly removing the endophyte with a fungicide application (unproven, but theoretically possible).

Current Research for Sports turf

Myerscough College and the University of Central Lancashire are investigating potential sustainable methods for sports turf management, and endophytes are a current area of research. The aim is to examine some of the fundamental questions and whether endophytes matter in sports turf management.

Using DNA analysis and immunoblot technology (plate 4 and 5) we have started detecting the presence of endophytes in UK sports turf, both in seed and surface.

The next stages will be to trial whether the infection has benefits or detriments for sports turf against the stresses of pests, disease and drought; and look at the effects of endophytes interaction with the immediate ecosystems of sports turf.

Endophytes could potentially be a solution towards sustainable turf management through a better understanding; and potentially reduce the application of harmful plant protection products and synthetic fertilisers.

Ultimately endophytes could bio-technically enhance the performance of sports turf surfaces, nevertheless, we need to know more about their ecology and our sports turf management towards them.

Acknowledgement: *Dr Andy Owen (Myerscough College)*