Endophytes – Fungal symbionts of grasses

Karen O'Hanlon PhD

Department of Agroecology, AU

Flakkebjerg

7th February 2012

AARHUS UNIVERSITY

Part 1 – General Introduction

Discovery of Grass endophytes

Endophytes

•Definition: An endophyte can be defined as an organism (bacterial, yeast or fungal) that lives within a plant.'

•Fungal endophytes are generally referred to as those fungi which asymtomatically colonise living tissues of plants, without causing any harm to the host.

•Fungal endophytes occur in all land plants (grasses, trees, shrubs, tropical trees etc.) and they are very diverse.

Endophytes

•Definition: An endophyte can be defined as an organism (bacterial, yeast or fungal) that lives within a plant.'

•Fungal endophytes are generally referred to as those fungi which asymtomatically colonise living tissues of plants, without causing any harm to the host.

•Fungal endophytes occur in all land plants (grasses, trees, shrubs, tropical trees etc.) and they are very diverse.

They can comprise many different species and have many different appearances.

•It is thought that the endophyte benefits from this relationship by gaining nutrients and shelter and seed-transmissal, while the benefits to the plant may be more indirect and complex

Why are endophytes so interesting?

AARHUS UNIVERSITY

- Endophytes provide benefits to their hosts

❑ **Pest resistance – probably the main driver in endophyte research**

❑ Bird deterrence – applications at airports to prevent birdstrike

❑ Tolerance to drought stress

❑ Improved salt tolerance

❑ Enhanced growth

❑ Increased resistance to disease

The Endophyte Life Cycle

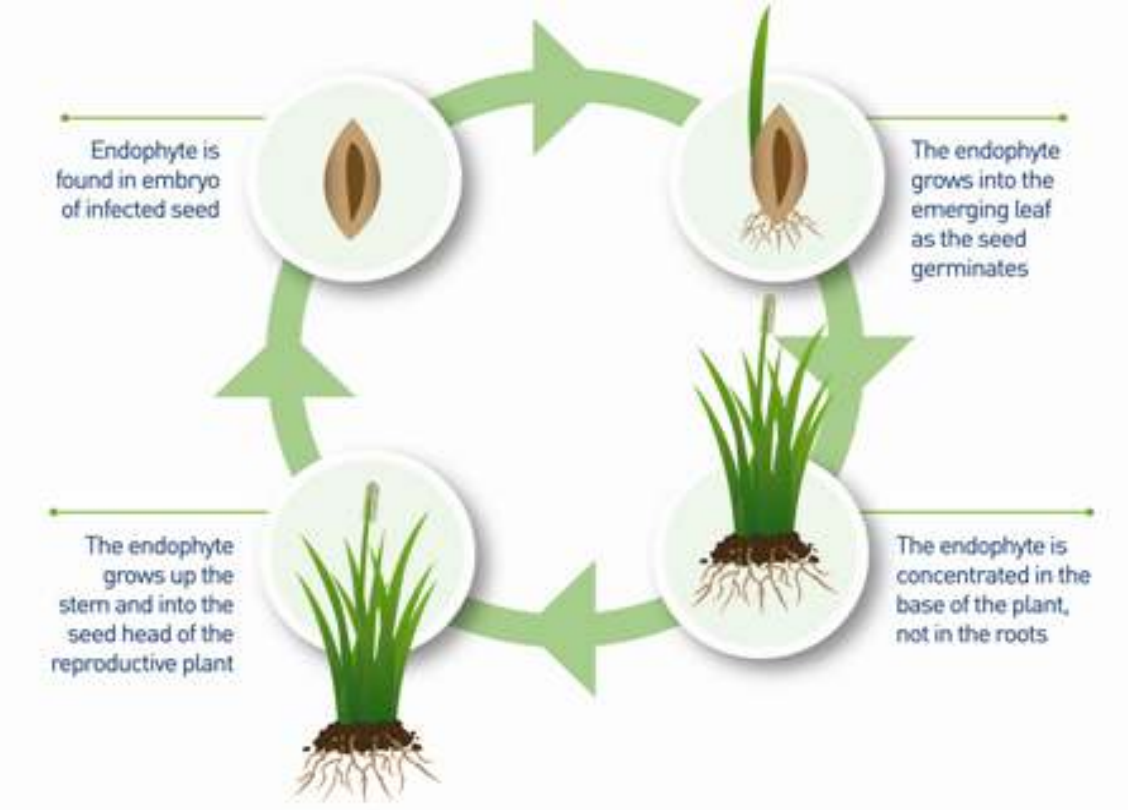


Image taken from
www.pggwrightsonseeds.com/technical/endophyte/

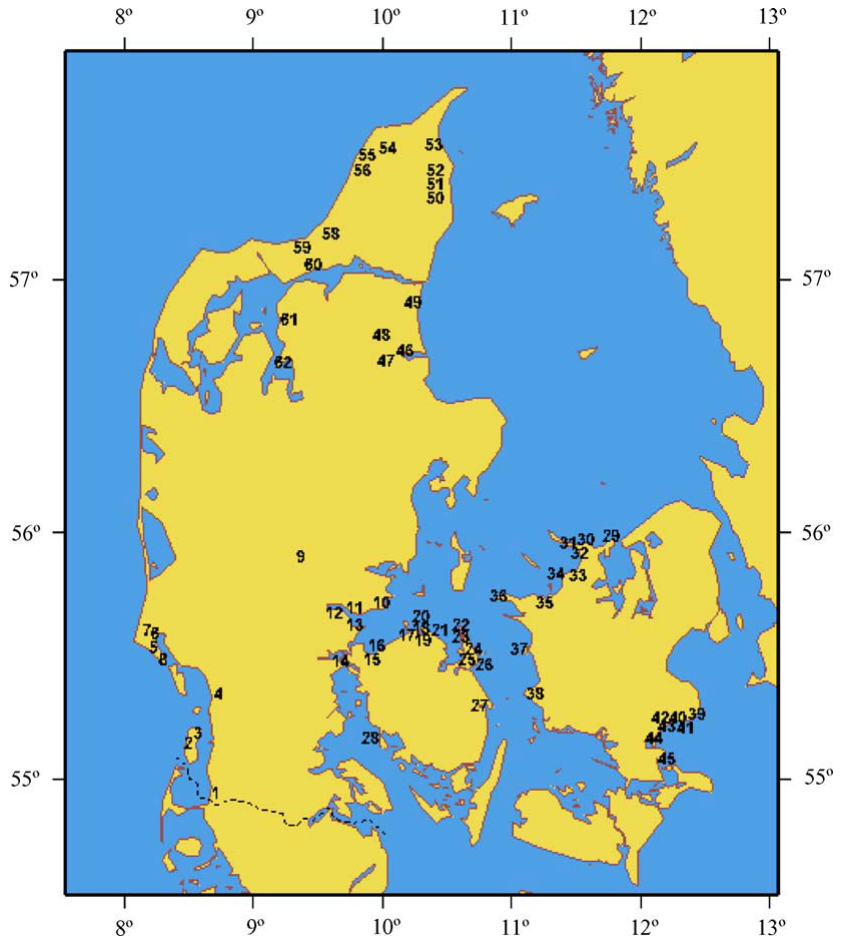
Neotyphodium Endophytes

- Seed-transmissible
- Closely related to the sexual *Epichloë* species
- *Epichloë* spp. cause symptoms known as choke disease in grasses (skedesvamp i hundgræs)
- Fungal spores produced around the leaf sheath during sexual stage lead to reduced flower and seed production (choke)
- *Neotyphodium* endophytes do not cause choke

Epichloë typhina on grass – image taken from www.wikipedia.org

Endophytes are also found in Denmark

- 62 Danish locations (12 habitat types) were investigated for the presence of the perennial ryegrass endophyte (*Neotyphodium lolli*)
- Most habitats were different types of semi-natural grassland
- Endophytes were found at 77 % of the locations, infection ranging from 4 to 82 %
- The highest infection rates were found in communities, which had been grazed or intensively used by the public
- *Neotyphodium* ryegrass endophytes are widely spread in semi-natural grass communities in Denmark – gives opportunities to exploit



Jensen and Rouland, 2004

The Endophyte Story

•Humans have known about endophytes for a long time, or at least known about the toxicities they cause

The earliest written record of forage grass toxicoses due to endophytes dates back to the biblical periods, approximately 50 A.D. (Matthew 13:25-401) in which the fungus seed of darnel (Lolium terndenturn L.) was indicated as being a noxious and toxic weed that caused problems for animals and humans[1]

•It is even thought that endophyte toxicosis is older, as darnel seed from 4,000 thousand year old archeological sites in Egypt contained the fungus!![2]

•The actual species of fungus found in the darnel at this time is still unknown, but is likely to be an unidentified *Neotyphodium*.

[1]Charles W. Bacon, Journal of Animal Science, 1995
[2] Tackholm and Tackholm, Flora of Egypt, Egyptian Uni. Press, 1941

How were endophytes discovered?

AARHUS UNIVERSITY

- 1942: Kentucky 31 tall fescue was released for pasture in the USA

How were endophytes discovered?

- 1942: Kentucky 31 tall fescue was released for pasture in the USA
- 1948: Fescue foot (crippling disease of cattle) emerged in New Zealand

How were endophytes discovered?

- 1942: Kentucky 31 tall fescue was released for pasture in the USA
- 1948: Fescue foot (crippling disease of cattle) emerged in New Zealand
- 1950 and onwards: Fescue foot described in the USA
- The full description of fescue toxicosis was defined in 1963 (including summer and winter symptoms)

How were endophytes discovered?

- 1942: Kentucky 31 tall fescue was released for pasture in the USA
- 1948: Fescue foot (crippling disease of cattle) emerged in New Zealand
- 1950 and onwards: Fescue foot described in the USA
- The full description of fescue toxicosis was defined in 1963 (including summer and winter symptoms)
- 1972: Kentucky 31 officially recognised as a cultivar due to demand for forage

How were endophytes discovered?

AARHUS UNIVERSITY

- 1942: Kentucky 31 tall fescue was released for pasture in the USA
- 1948: Fescue foot (crippling disease of cattle) emerged in New Zealand
- 1950 and onwards: Fescue foot described in the USA
- The full description of fescue toxicosis was defined in 1963 (including summer and winter symptoms)
- 1972: Kentucky 31 officially recognised as a cultivar due to demand for forage
- During early descriptions of fescue foot, ergot alkaloids were isolated from toxic tall fescue, even though the classical ergot fungus (*Claviceps*) was not present…

How were endophytes discovered?

AARHUS UNIVERSITY

- 1942: Kentucky 31 tall fescue was released for pasture in the USA
- 1948: Fescue foot (crippling disease of cattle) emerged in New Zealand
- 1950 and onwards: Fescue foot described in the USA
- The full description of fescue toxicosis was defined in 1963 (including summer and winter symptoms)
- 1972: Kentucky 31 officially recognised as a cultivar due to demand for forage
- During early descriptions of fescue foot, ergot alkaloids were isolated from toxic tall fescue, even though the classical ergot fungus (*Claviceps*) was not present…
- 1973: Fungal species were isolated from toxic tall fescue pasture in Georgia (Robbins, Porter , Bacon)

How were endophytes discovered?

AARHUS UNIVERSITY

- 1942: Kentucky 31 tall fescue was released for pasture in the USA
- 1948: Fescue foot (crippling disease of cattle) emerged in New Zealand
- 1950 and onwards: Fescue foot described in the USA
- The full description of fescue toxicosis was defined in 1963 (including summer and winter symptoms)
- 1972: Kentucky 31 officially recognised as a cultivar due to demand for forage
- During early descriptions of fescue foot, ergot alkaloids were isolated from toxic tall fescue, even though the classical ergot fungus (*Claviceps*) was not present…
- 1973: Fungal species were isolated from toxic tall fescue pasture in Georgia (Robbins, Porter, Bacon)
- 1973-77: Toxicological studies revealed that these fungi were toxic & produced ergot alkaloids

How were endophytes discovered?

•1942: Kentucky 31 tall fescue was released for pasture in the USA

•1948: Fescue foot (crippling disease of cattle) emerged in New Zealand

•1950 and onwards: Fescue foot described in the USA

•The full description of fescue toxicosis was defined in 1963 (including summer and winter symptoms)

•1972: Kentucky 31 officially recognised as a cultivar due to demand for forage

•During early descriptions of fescue foot, ergot alkaloids were isolated from toxic tall fescue, even though the classical ergot fungus (*Claviceps*) was not present…

•1973: Fungal species were isolated from toxic tall fescue pasture in Georgia (Robbins, Porter, Bacon)

•1973-77: Toxicological studies revealed that these fungi were toxic & produced ergot alkaloids

•1977: Charles Bacon and co. suggested that *Epichloë typhina* was responsible for the cattle toxicity in toxic tall fescue pasture grass.

Endophytes produce toxins

AARHUS UNIVERSITY

•The association between endophyte presence and tall fescue toxicosis was then confirmed by others and so it became accepted that toxic endophytes cause livestock toxicosis

•After some discussions, it was agreed that the identity of the tall fescue endophyte was *Acremonium coenophialum* (now known as ***Neotyphodium coenophialum***)

•In the early 1980's, a similar discovery was made about perennial ryegrass staggers, which were found out to be caused by the perennial ryegrass endophyte, *Neotyphodium lolli*

Today we know that: (in general)

- **Tall fescue – *N. coenophialum***
- **Meadow fescue – *N. uncinatum***
- **Perennial ryegrass – *N. lolli***
- **Red fescue – *E. typhina***

AARHUS UNIVERSITY

Part 2 –

The Benefits of Grass Endophytes

Endophytes provide benefits to their hosts

- **Pest resistance – currently undergoing a lot of work**
- Bird deterrence – applications at airports to prevent birdstrike
- Tolerance to drought stress
- Improved salt tolerance
- Enhanced growth
- Increased resistance to disease

……this had led to the **commercialisation of 'novel endophytes'**

Novel means that the endophyte is not naturally occuring in the cultivar
Wild-type means that the endophyte is naturally occuring in the cultivar

What toxins are produced?

5 classes of toxins (secondary metabolites) produced in varying amounts by different endophytes.

1. **Lolitrems** – animal toxic, ryegrass staggers

2. **Ergot alkaloids** (ergovaline) – but animal toxic, fescue toxicosis

1. **Peramine – anti-insect**

2. **Loline alkaloids* – anti-insect, root protection?**

3. **Epoxy-janthitrems** - resistance to a wide range of insect pests, but may cause ryegrass staggers

Commercially used novel endophytes

Endophyte	Source	Potential Toxin Production Lol B	Ergo	Pera	Loline	Jant	Pest Resistance	Animal Friendly?
Standard 'wild-type'	*N. lolli*	+++	+++	+++	-	-	Argentine stem weevil Pasture mealy bug. Black beetle larvae and adults.	☹
AR1	*N. lolli*	-	-	+++	-	-	Argentine stem weevil. Pasture mealy bug. Variable control of root aphid. Mild deterrence to black beetle adults.	☺ currently the only safe endophyte for horses, deer and alpacas.
Endo 5 (AR6) **NEA2**	*N. lolli*	- +	+ +	++ ++	- -	- -	Argentine stem weevil. Pasture mealy bug. No control of black beetle larvae but some control of the adult.	😐 Avoid grazing fresh regrowth during summer and autumn.
AR37	*N. lolli*	-	-	-	-	++++	Argentine Stem weevil larvae. Good control of pasture mealy bug and root aphid. Control of porina caterpillar. Provides no control of black beetle larvae but some control of the adult.	☹
AR542 / MAXP (Q)	*N. coenophialum*	-	-	++	+	-	Provides low to moderate control of Argentine stem weevil and black beetle. Good control of porina caterpillar. Small deterrence effect on grass grub feeding. Provides resistance to pasture mealy bug and has a small effect on root aphid.	☺
E34 (Barenbrug)	Probably *N. coenophialum*						High forage yields and persistent under varying climatic conditions	☺

Information in this table adapted from http://www.cropmark.co.nz

Insect Protection

Adults

Larvae

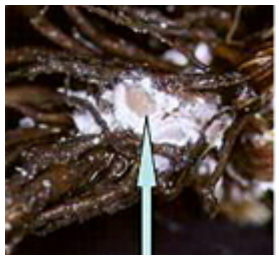
Eggs

Perennial ryegrass

Black beetle

Larvae

Grass grub



Pasture Mealy Bug

Porina

Root Aphid

Image from www.grasslanz.com

Which endophytes produce which toxins?

•4 classes of toxins (secondary metabolites) produced in varying amounts by different endophytes.

Toxin Family	Endophyte	Effects
Lolitrems	Perennial Ryegrass (*Neotyphodium lolli*)	Lolitrem B – causes ryegrass staggers. **Seasonal and yearly variations (usually high in summer and autumn).** Growth conditions, N,moisture can influence. Hay can be problematic, dilution a solution
Ergot Alkaloids (EA)	Tall Fescue (*N. coenophialum*) Perennial Ryegrass (*N. lolli*)	Ergovaline – fescue foot, summer ill thrift, reproductive problems. Hyperthermia, lower feeding, weight loss.
Peramine	Tall Fescue Perennial Ryegrass (as for EA)	No adverse effects on animals
Loline Alkaloids	Tall fescue (*N. coenophialum*) Some annual ryegrasses Meadow fescue *N. uncinatum* and *N. siegelii*	No adverse effects on animals May be high producers of lolines – they are interesting as they have the potential for biocontrol against root-eating insects

AARHUS UNIVERSITY

Part 3 – Researching Endophytes

Detecting *Neotyphodium* endophytes – Microscopy

AARHUS UNIVERSITY

- Seed-transmissible and can be found in leaf sheaths and seeds

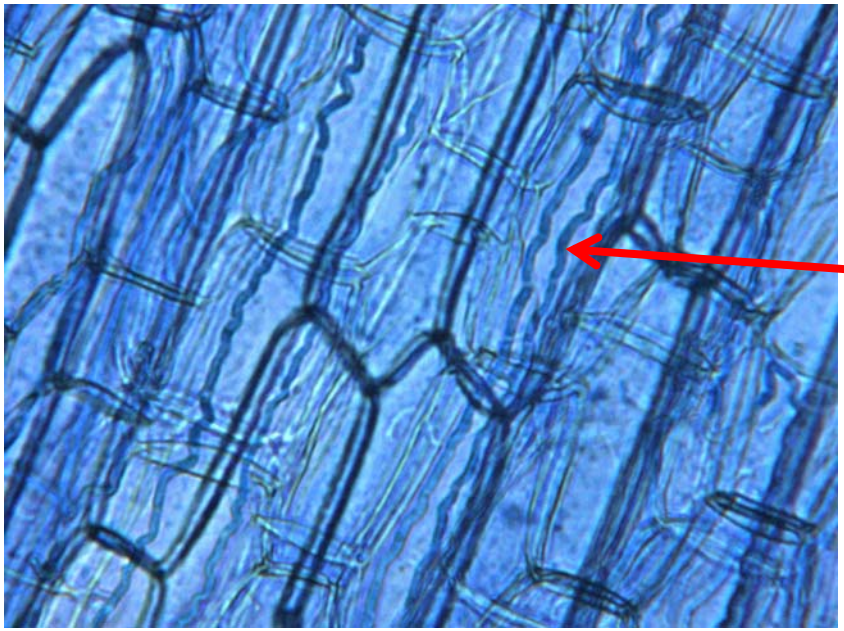
Detecting *Neotyphodium* endophytes – Microscopy

AARHUS UNIVERSITY

•Seed-transmissible and can be found in leaf sheaths and seeds

•Leaf sheath staining with aniline blue reveals convoluted fungal hyphae which grow along the longitudinal leaf axis

•Seed staining reveals fungal hyphae amongst the squashed seed cells



Leaf Staining

Fungal hyphae 'curly lines'

Note: Seeds can also be stained in a similar manner

Image: AU, Flakkebjerg

Measuring the amount of endophyte – Considerations

AARHUS UNIVERSITY

- Measuring the amount of endophyte and toxin is clearly important

- One way is simply to count the proportion of tillers/seeds that contain fungal material

  BUT

  - Some plants can have a lot of endophyte
  - While others will have less endophyte ……..but they are both called E+

- Also, the same plant can have infected and uninfected parts

- Measuring the actual amount of endophyte is difficult and very expensive

- Usually taking the proportion is enough to describe 'how severe' an infection is

- Measuring the toxins may be more important than measuring the endophyte

- Many different methods exist and right now these need to be done in a laboratory setting

What does this mean for farmers:

- Several important questions remain unanswered:
  - When exactly are the plants most toxic to grazing animals?
  - Can regrowth be used for grazing?
  - Can straw be used for grazing?

What does this mean for farmers:

- Several important questions remain unanswered:
  - When exactly are the plants most toxic to grazing animals?
  - Can regrowth be used for grazing?
  - Can straw be used for grazing?

- It is thought that:
  - Toxin concentrations increase in higher temperatures (late summer/autumn)
  - Toxins and endophytes are more prevalent at the base of the plant

- But the truth is that we don't have all the answers!

Endophyte Research – the future

Many outstanding questions about the endophyte life cycle and the endophyte-plant relationship:

1)How does the endophyte travel within the plant, and how does this effect the level of endophyte infection

2)What factors are involved in the stability of the endophyte in the seed?

3)Can we use high loline producing endophytes to protect against root-eating insects of ryegrass?

Endophyte Research at Flakkebjerg

AARHUS UNIVERSITY

1) **Improving the transfer of endophytes into new hosts**

- For example: transfer meadow fescue endophytes into perennial ryegrass
- Understanding the interaction between the endophyte and the plant is important

2) **Wheat Endophyte Project**

- Wheat and other cereals are challenged by serious fungal diseases e.g. Fusarium head blight
- Searching for endophytes in wheat populations
- Looking for endophytes with an effect against fungal diseases (biocontrol)

AARHUS
UNIVERSITY

Thank you!!