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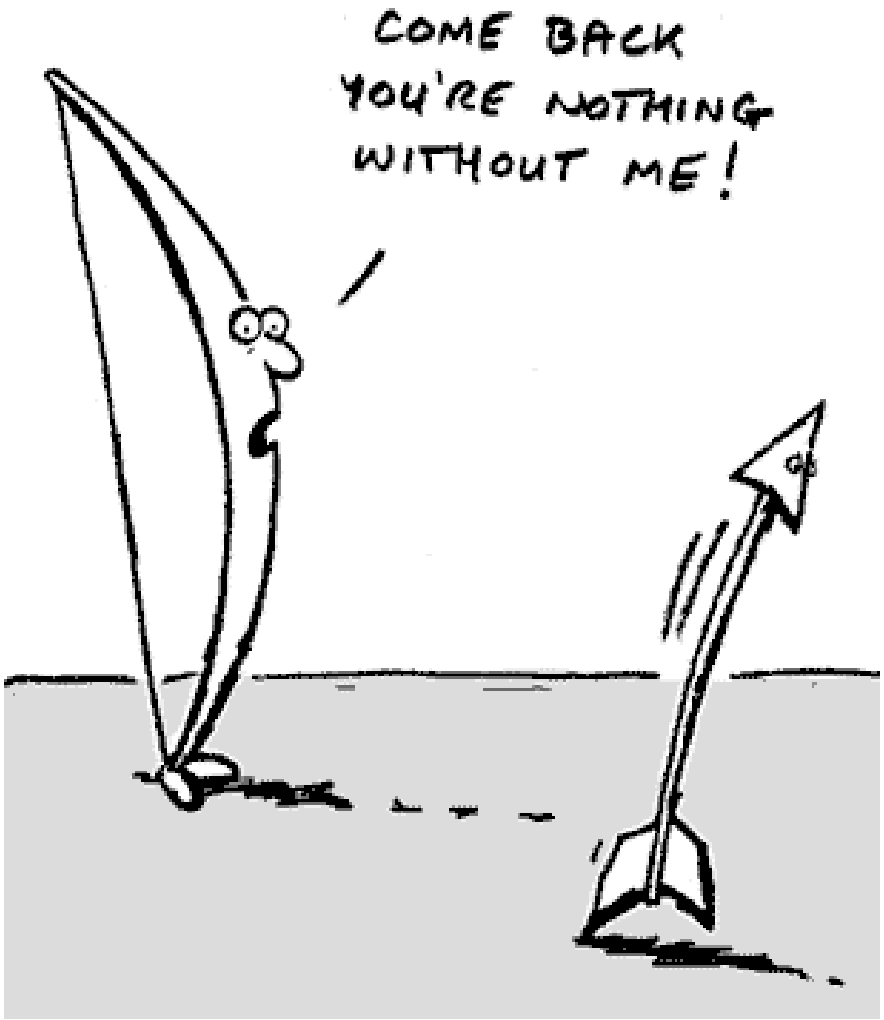
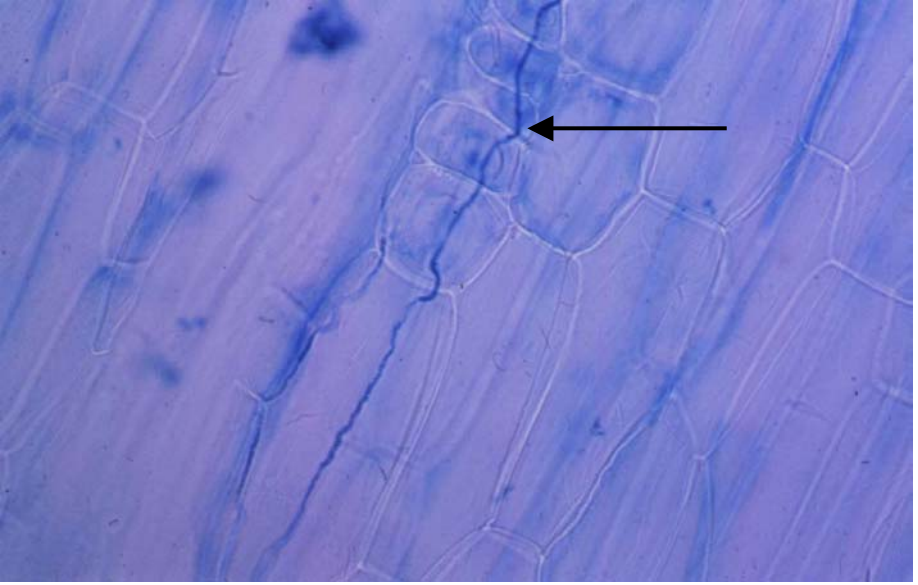
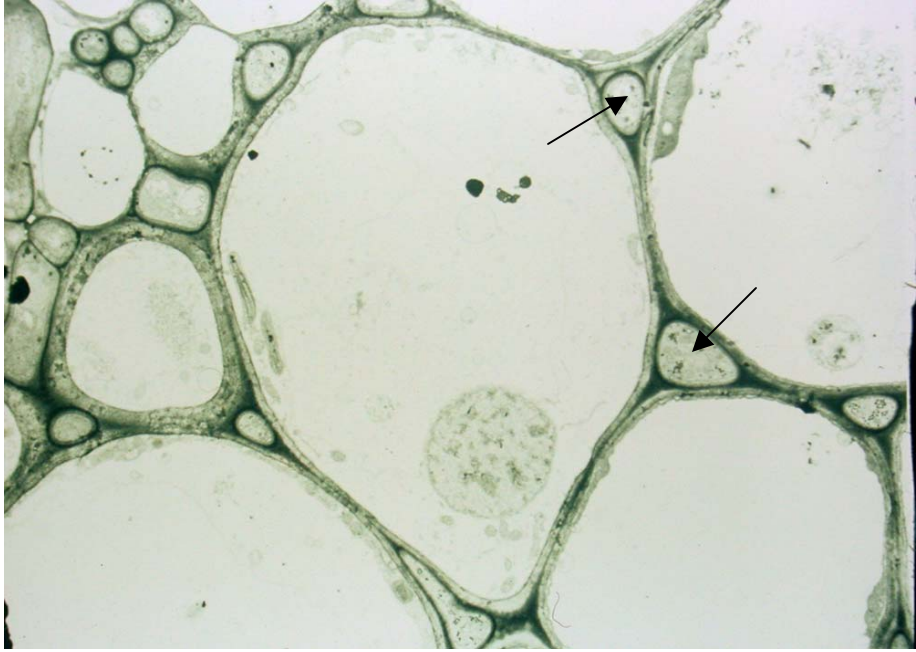
USDA ars

http://www.tmru.saa.ars.usda.gov/glenn/glenn.html

FUNGAL
ATTACK
SR 93

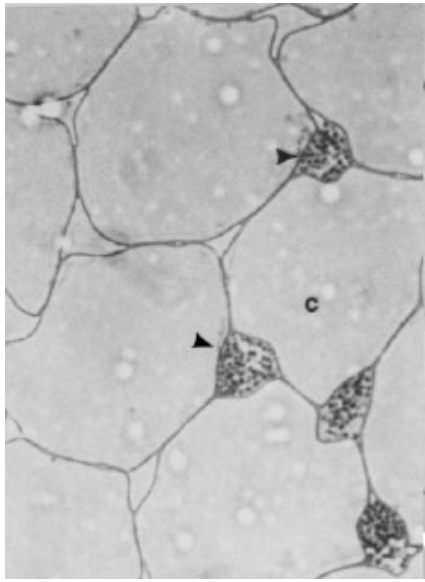

COME BACK
YOU'RE NOTHING
WITHOUT ME!





Endophytes

- Symbiotic fungi or bacteria living within plant tissues; typically in intercellular spaces; for most if not all of their life cycle
- Usually systemic, but can be limited to localized infections
- All levels of plant tissue can be infected (roots, stems, leaves); depends upon association
- Typically cause little or no disease
- Often a mutualistic association



Grass Endophytes

- Endophytes of grasses and sedges; limited to family Clavicipitaceae (Hypocreales)
- Systemic throughout aboveground tissues (not in roots)
- Often seed transmitted (vertical transmission)
- Produce a variety of secondary metabolites (mycotoxins)
- Generally only one fungal genotype per plant

Other Endophytes

- Pyrenomycetes; mainly members of Xylariales
- Broad host range (angiosprems and conifers)
- “Woody endophytes”
- Tend to be more localized infections
- Spore dispersal (horizontal transmission)
- One leaf or one plant may contain dozens or hundreds of distinct fungal species

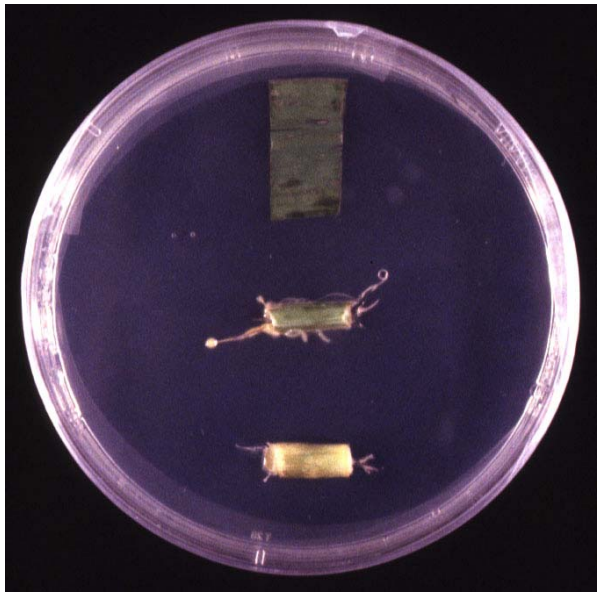
Endophytic vs. Latent Infections

- Endophytic infection
  - Fungus forms inconspicuous, mostly intercellular, long-term associations with host; generally symptomless; hyphae are viable, growing, and biochemically active.
  - "…endophytism is both a life habit and a biological association with ecological and physiological relevance."

- Latent or dormant infection
  - Fungus forms intercellular/intracellular infections; transiently symptomless due to dormancy; hyphae may be viable but are not actively growing.
  - A symptomless stage of an infection that can become actively pathogenic when conditions are conducive.

A Broad Definition of "Endophyte"

- Functional definition of "endophyte"
  - Recovery from surface-sterilized plant material
  - Microscopically demonstrated to reside within plant tissue



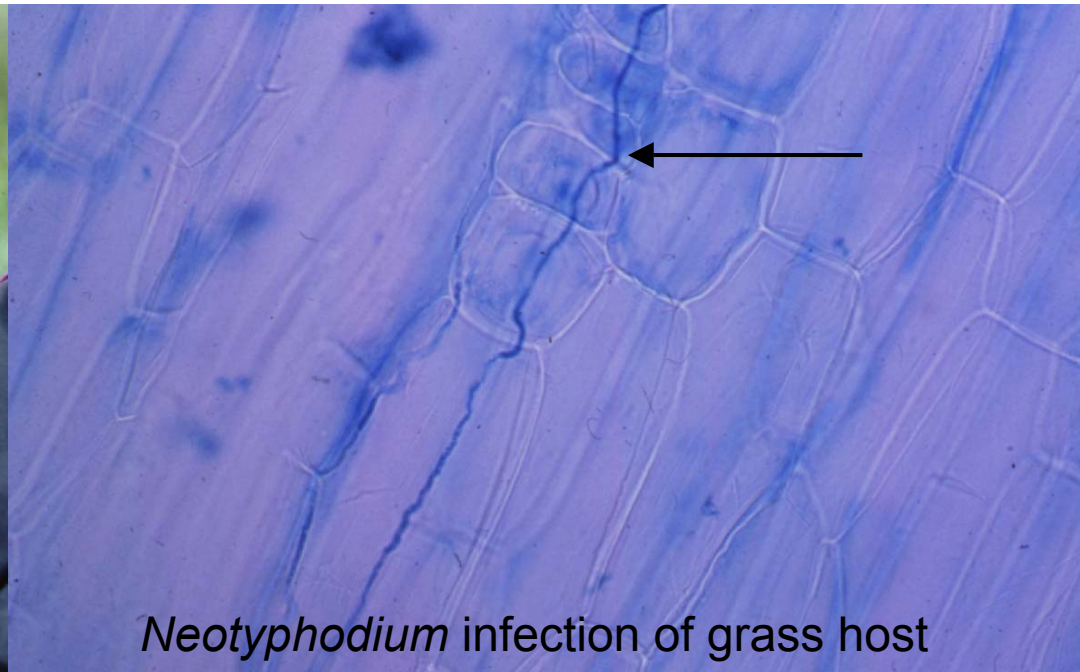
Endophytic vs. Epibiotic Fungi

- Endophytic (endobiotic) fungi
  - Are mostly intercellular, forming long-term associations with host; derive nutrients from intercellular spaces (no haustoria); may form external reproductive stromata.

- Epibiotic fungi
  - Highly evolved, host-specific, intimate associations with their hosts, but are restricted to external surfaces.
  - No systemic intercellular hyphae.
  - Form reproductive stromata on surface of plant leaf or inflorescence.
  - Modify cuticle layer and epidermal cells to facilitate nutrient acquisition (no haustoria).

Grass Endophytes/Epibionts

Fungi (kingdom)
- Ascomyctoa (phylum)
  - Sordariomycetes (class)
    - Hypocreales (order)
      - Clavicipitaceae (family)
        - *Epichloë*/*Neotyphodium* (endophytes)
        - *Balansia* (endophytes & epibionts)
        - *Claviceps* (epibionts)
        - *Myriogenospora* (epibionts)



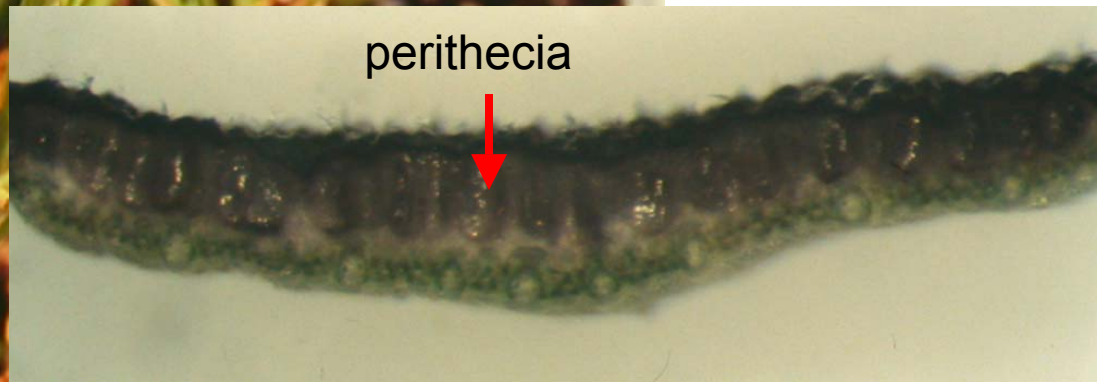
Neotyphodium infection of grass host

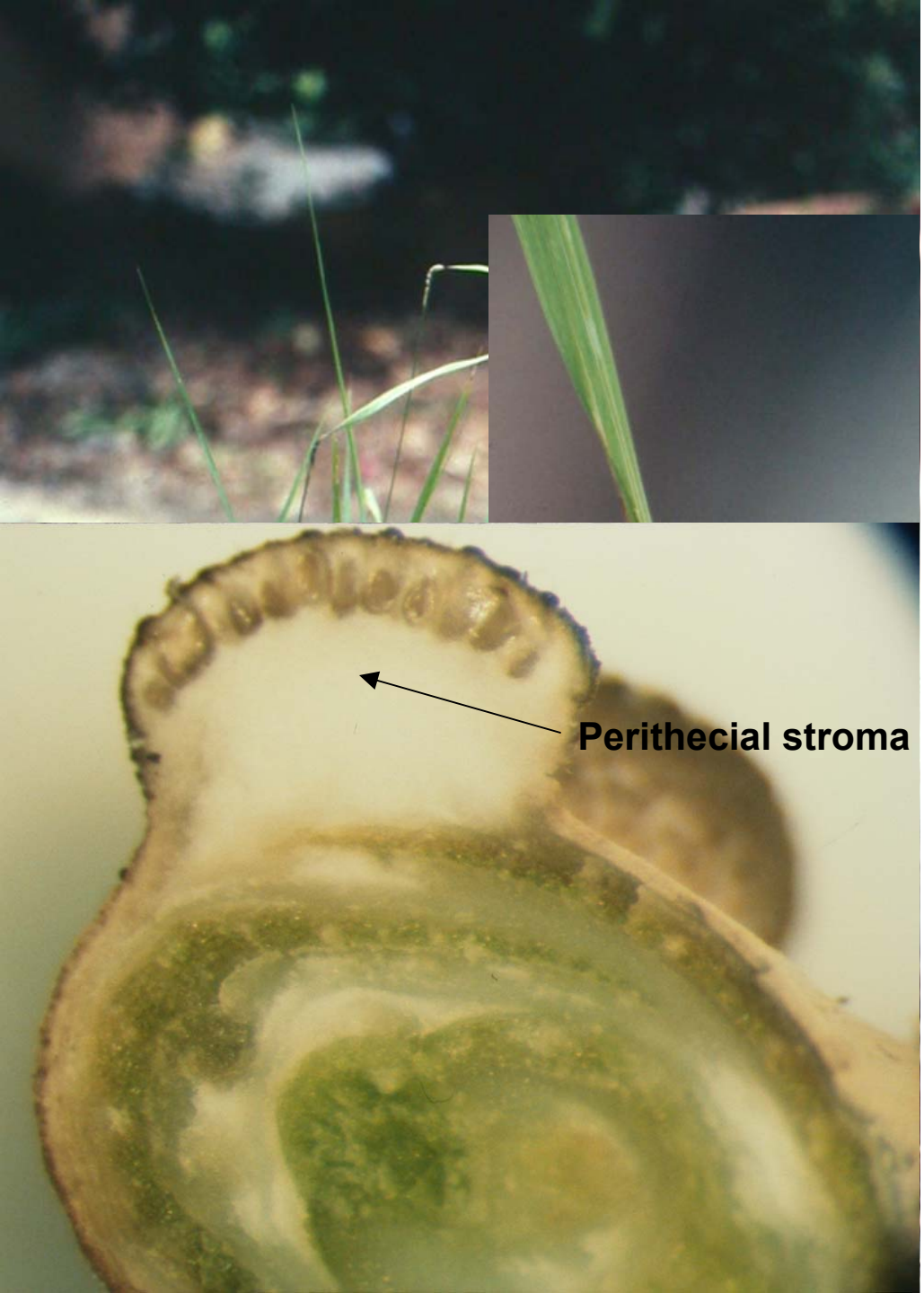
Claviceps purpurea

Epibiotic, localized infection of floret ovary; replaces tissue of the ovule; replaces kernel with a sclerotium

Balansia epichloë
(on *Sporobolus poiretii)*

Endophytic with perithecial stroma produced on upper surface of leaf





Balansia pilulaeformis
(on *Chasmanthium laxum)*

Epibiotic with hypothallus enveloping and aborting the inflorescence

No systemic, intercellular hyphae

Cushion-shaped perithecial stromata produced on a hyphothallus

Myriogenospora atramentosa
(on *Ichnanthus pallens*)

Epibiotic with perithecial stroma developing on leaf surface; often folding or rolling the leaf.

No systemic, intercellular hyphae

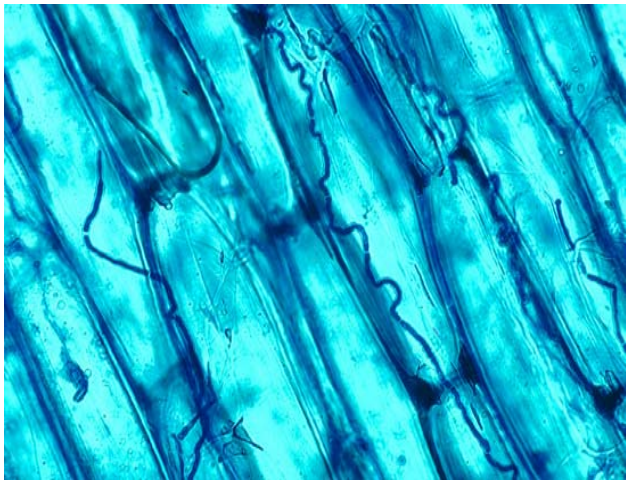
Linear stromata with single row of perithecia

Myriogenospora atramentosa
(on *Andropogon & Erianthus spp.*)

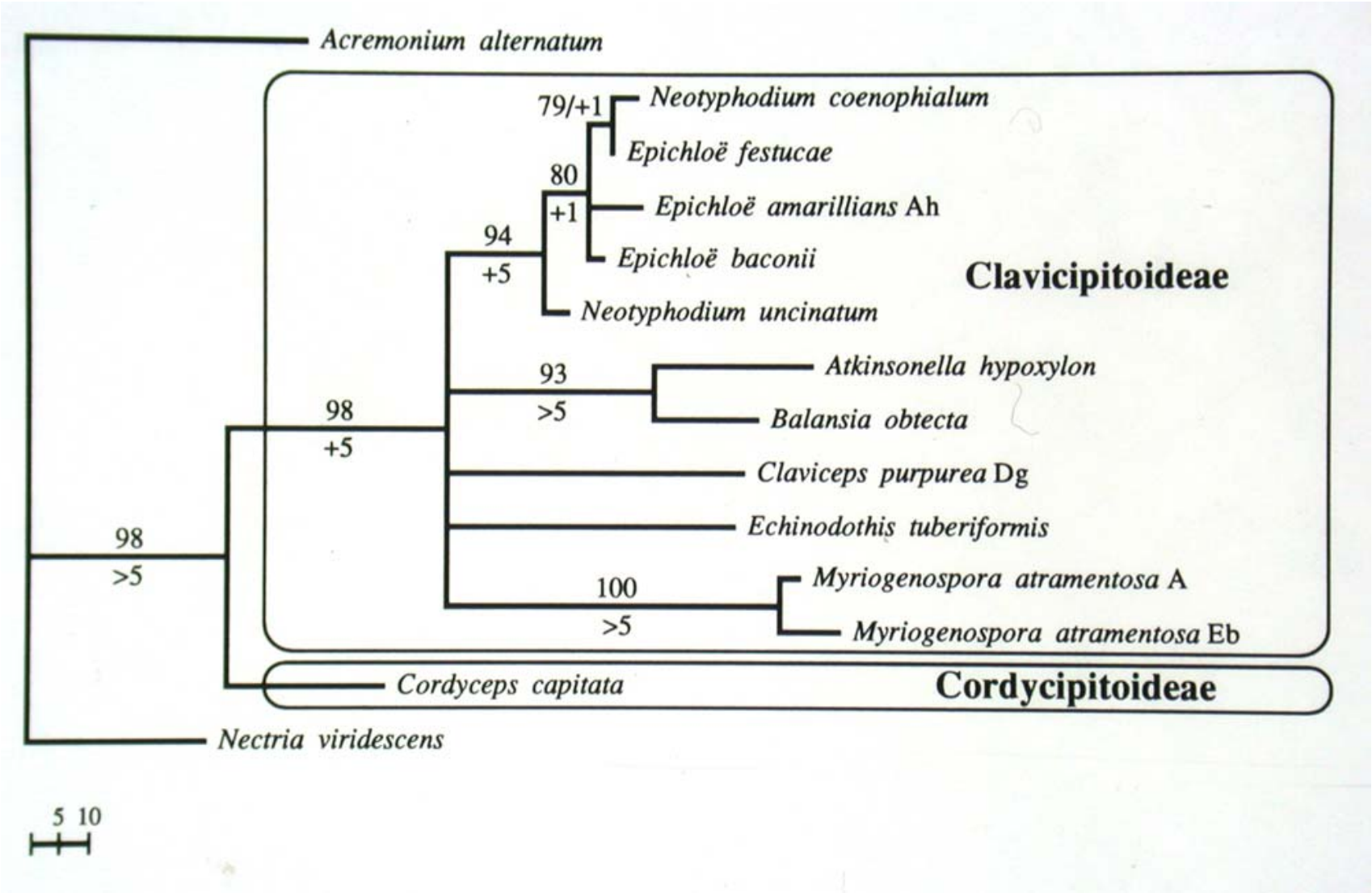
Epichloë & Neotyphodium

(on various species of grasses *)*

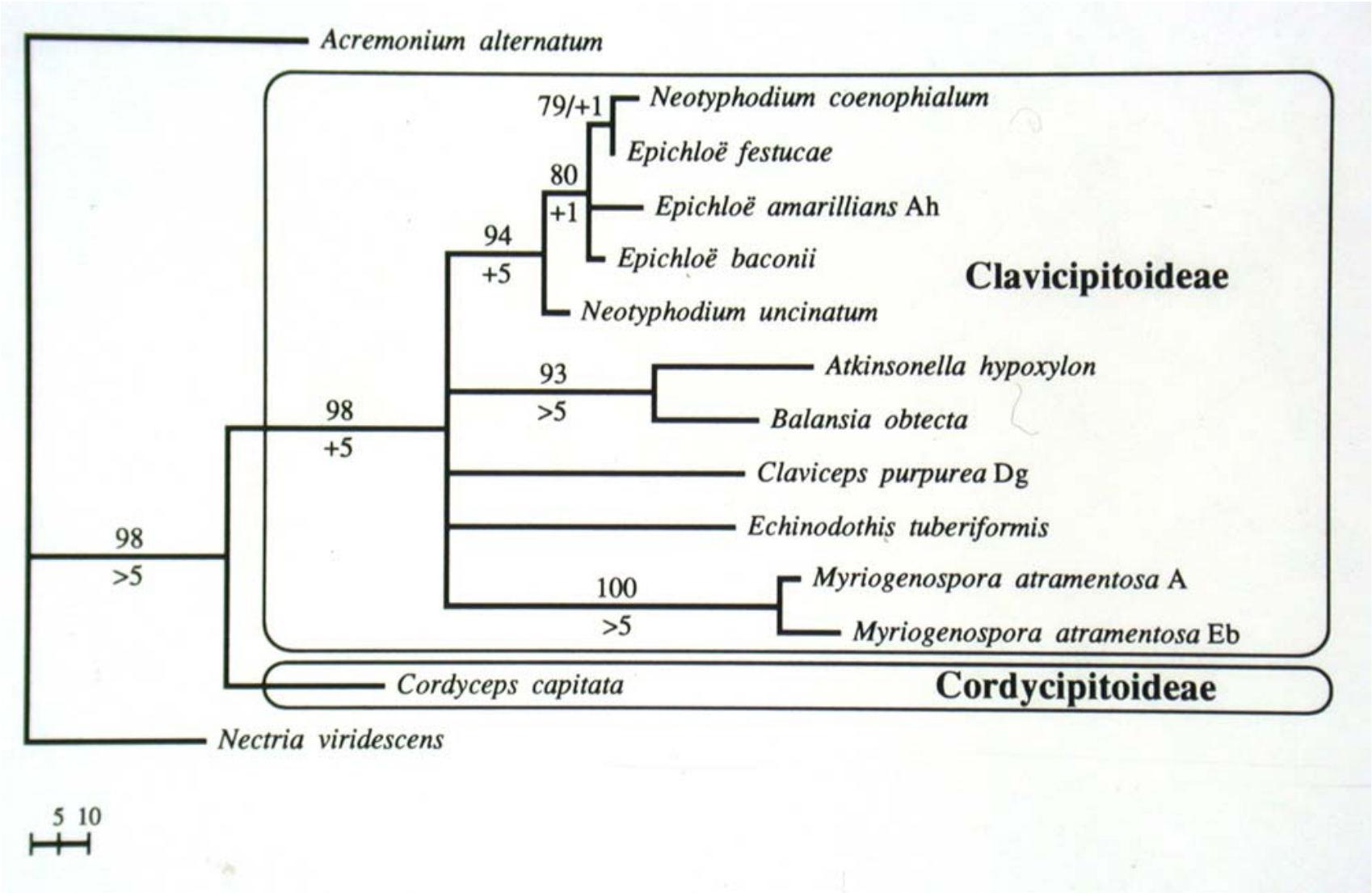
Endophytic with conidial/perithecial stroma enveloping and aborting the inflorescence (choke disease)



Grass associated genera are within family Clavicipitaceae subfamily Clavicipitoideae



Asexual *Neotyphodium* endophytes are derived from sexually reproducing *Epichloë*

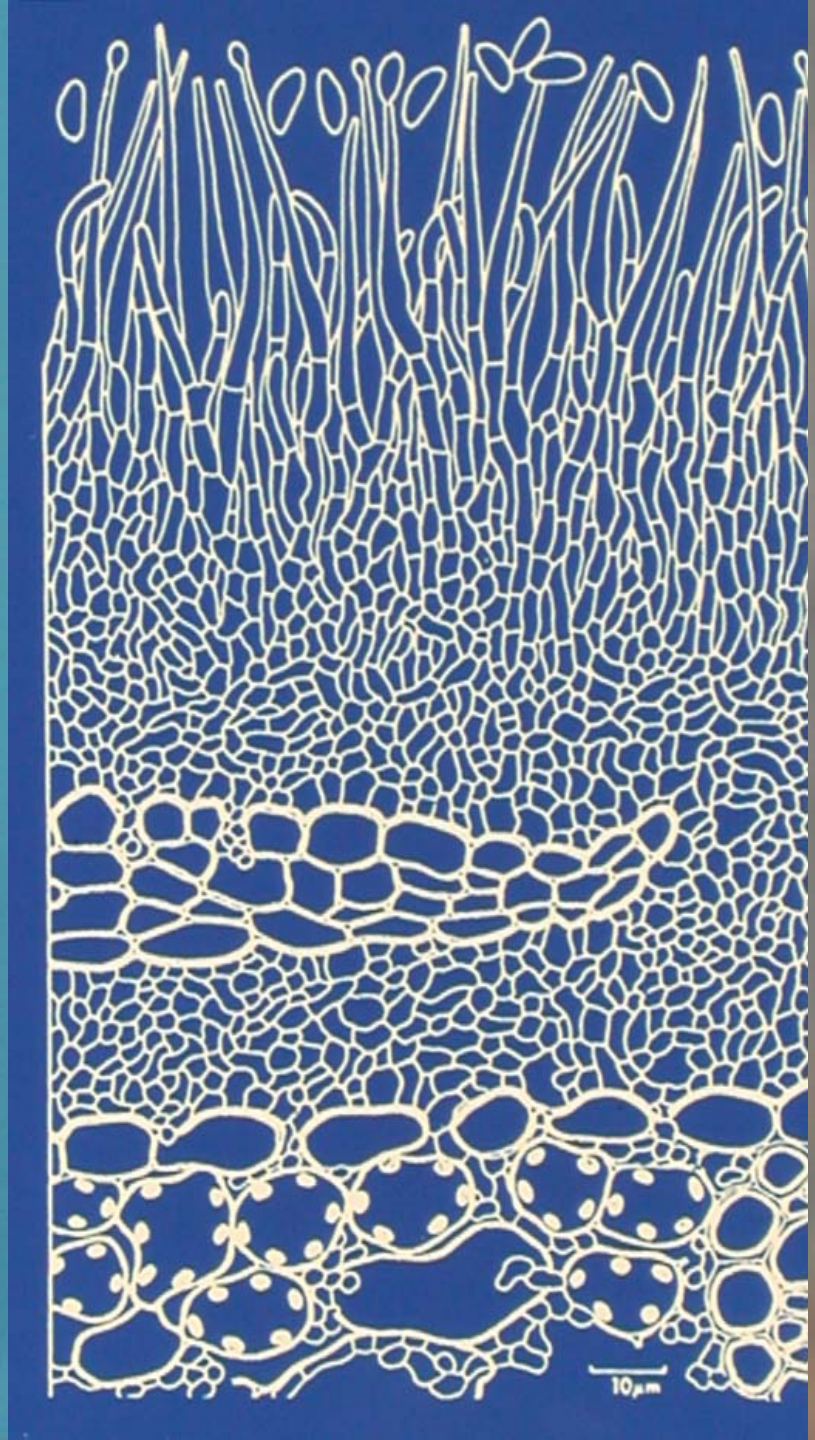


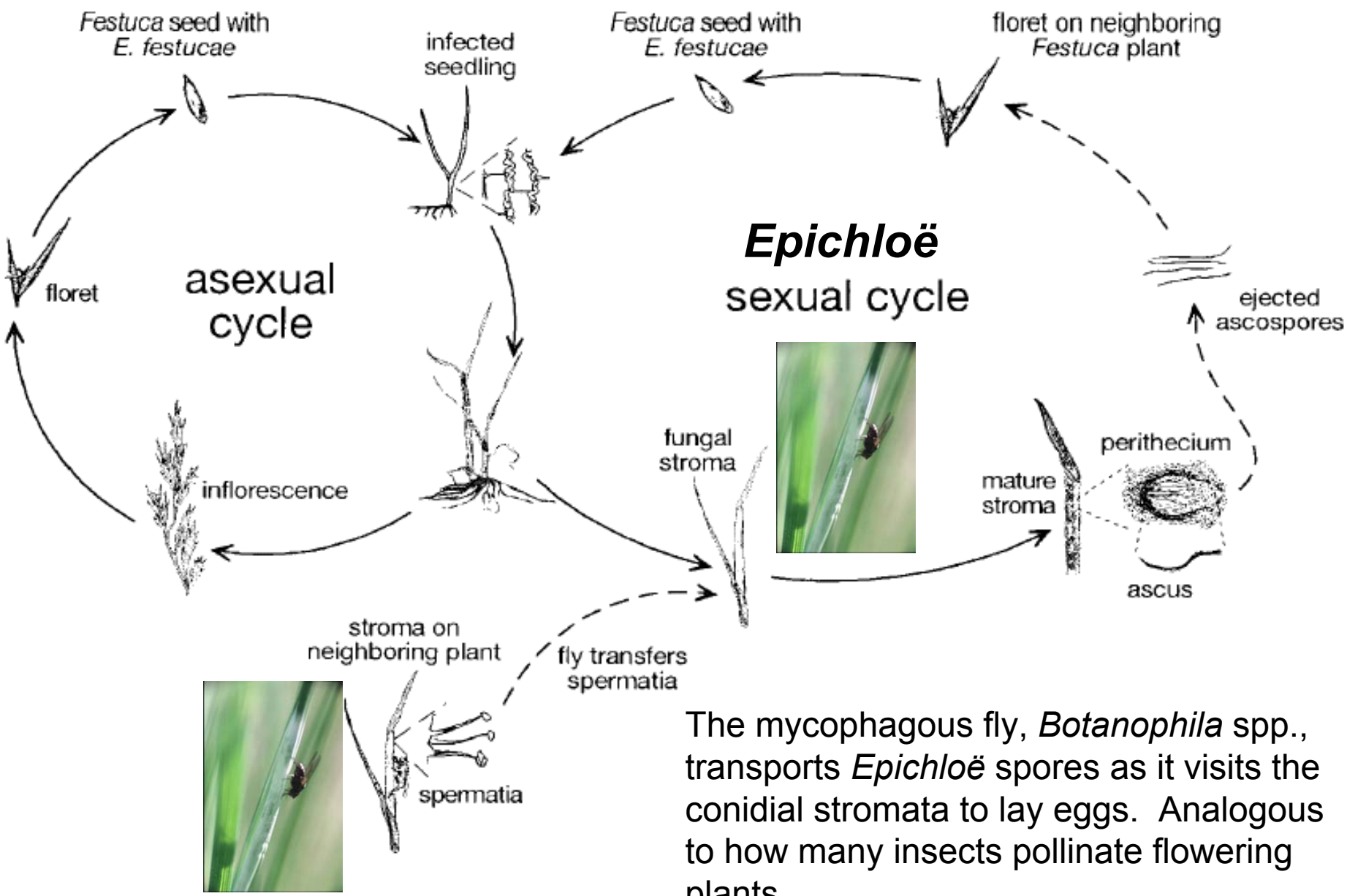
Sexual *Epichloë* endophytes

Asexual *Neotyphodium* endophytes

Table 1: Contrasting characteristics of Types I, II, and III grass/endophyte associations

	Symptomatic (Type I)	Mixed (Type II)	Asymptomatic (Type III)
Fungus:			
Reproduction	Sexual	Both	Clonal
Transmission	Horizontal	Both	Vertical
Propagule	Ascospores	Both	Seeds
Host:			
Reproduction	Sterile/clonal	Partial sterility	Sexual
Interaction	Pathogenic	Intermediate	Mutualistic
Infection frequency	Low-moderate	Intermediate	High
Taxonomy	Entire grass family	C3 pooid grasses	C3 pooid grasses


10μm



The mycophagous fly, *Botanophila* spp., transports *Epichloë* spores as it visits the conidial stromata to lay eggs. Analogous to how many insects pollinate flowering plants.

Sexual *Epichloë* endophytes

Asexual *Neotyphodium* endophytes

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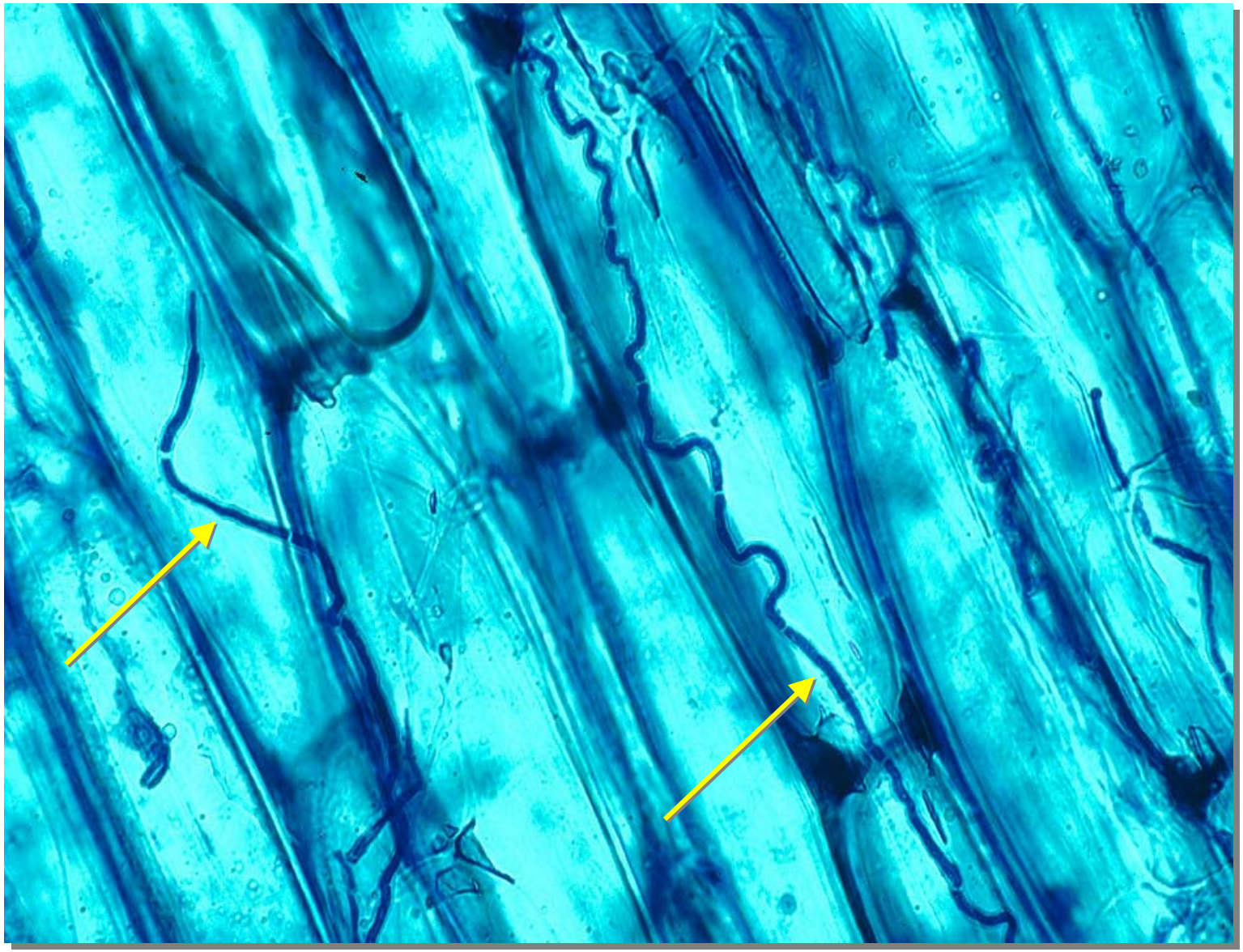
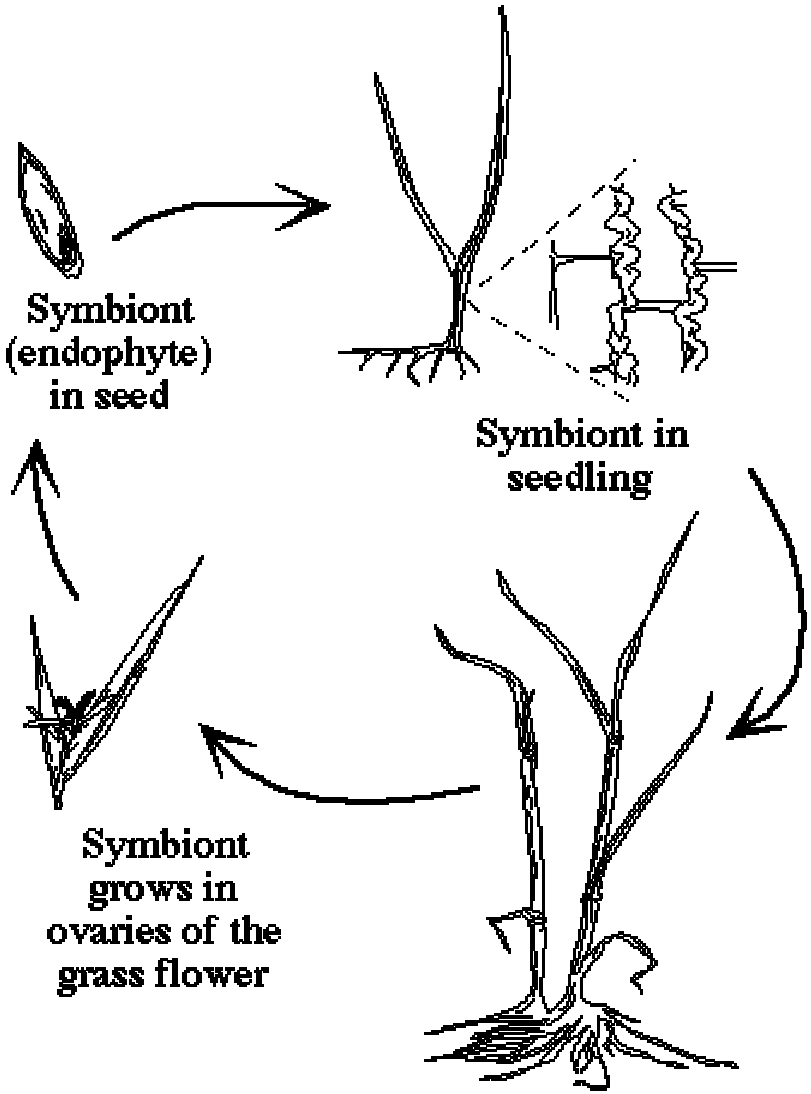


Neotyphodium coenophialum **= archetypical endophyte**

(in tall fescue leaf sheath)

Vertical (seed) transmission of *N. coenophialum*



Tall fescue seed infected with *N. coenophialum*

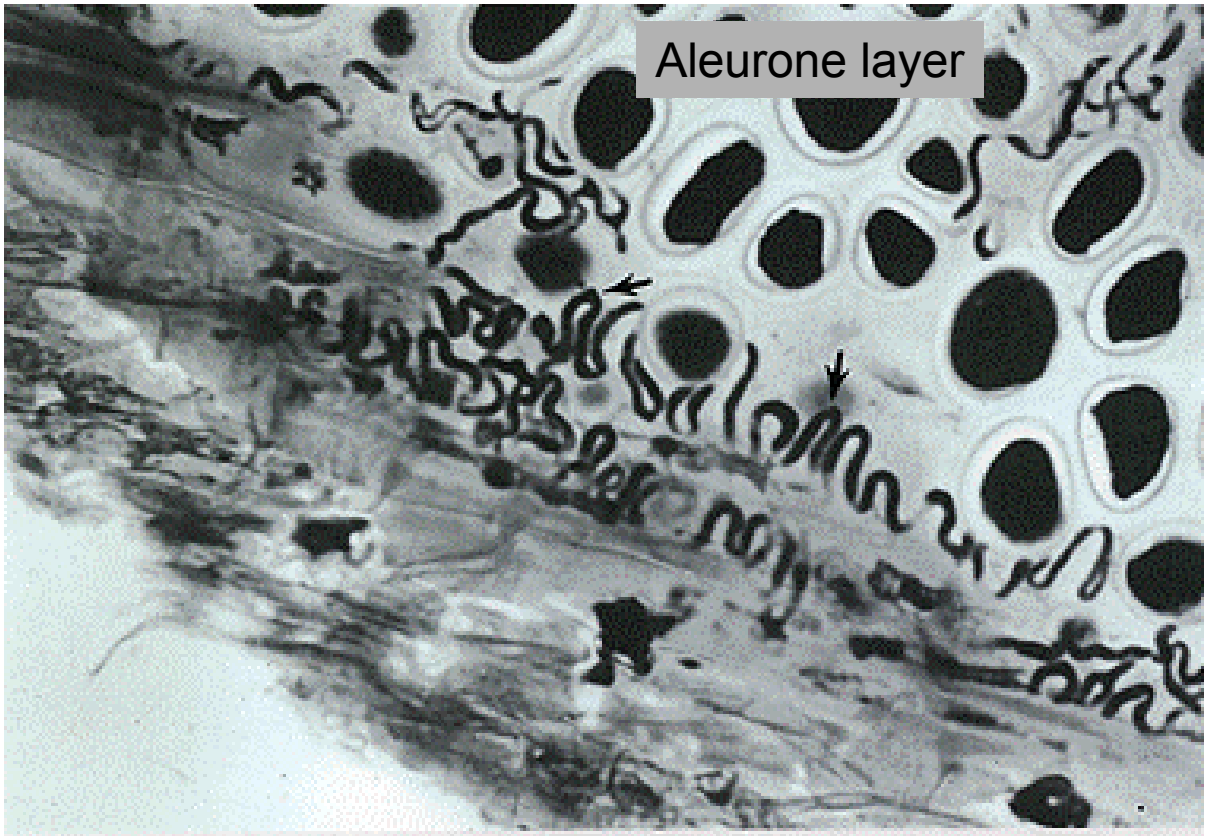
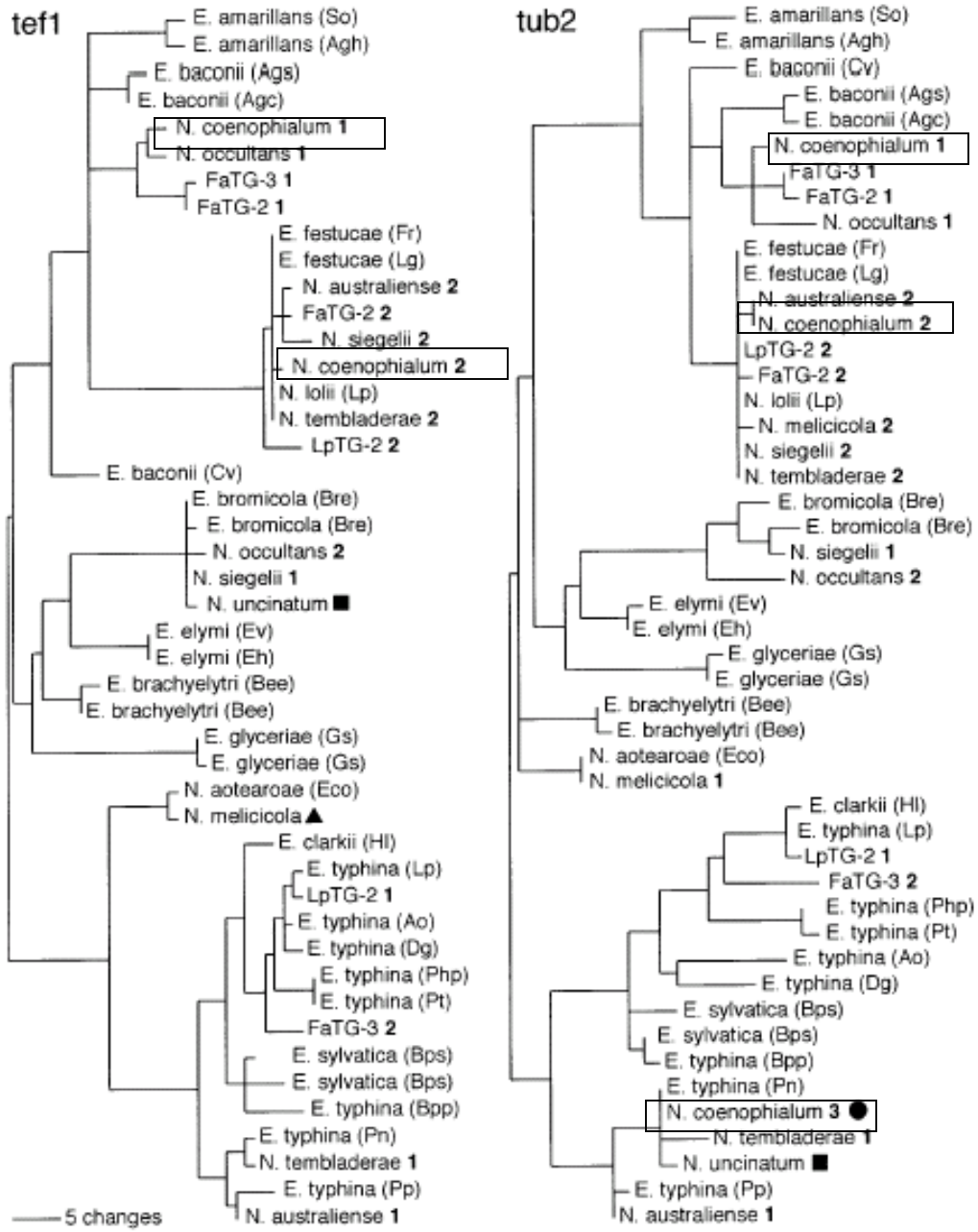


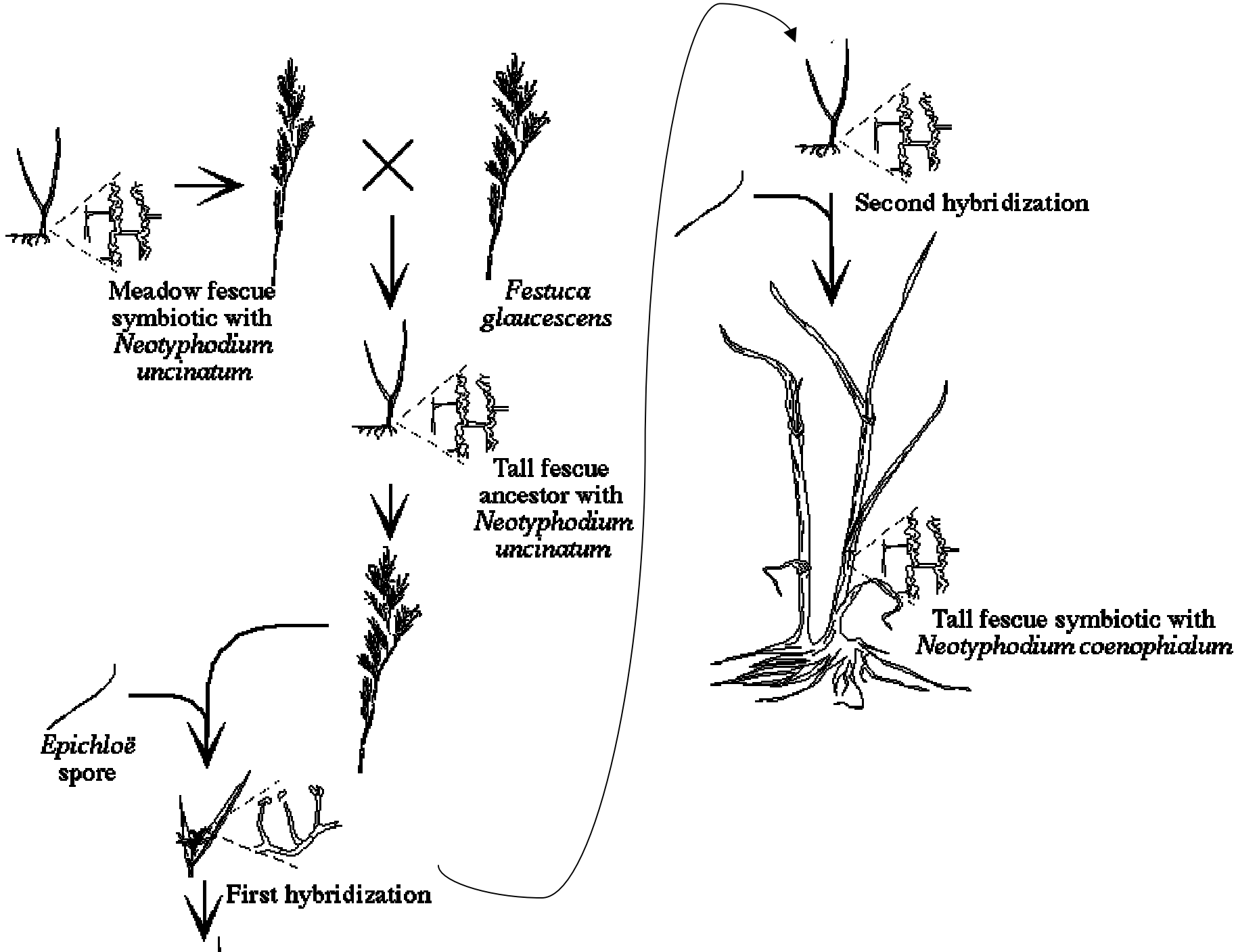
Fig. 2. Tangential section through seed of *Festuca arundinaceae* showing mycelium (arrows) within seed (X 800)

Many *Neotyphodium* species are interspecies hybrids

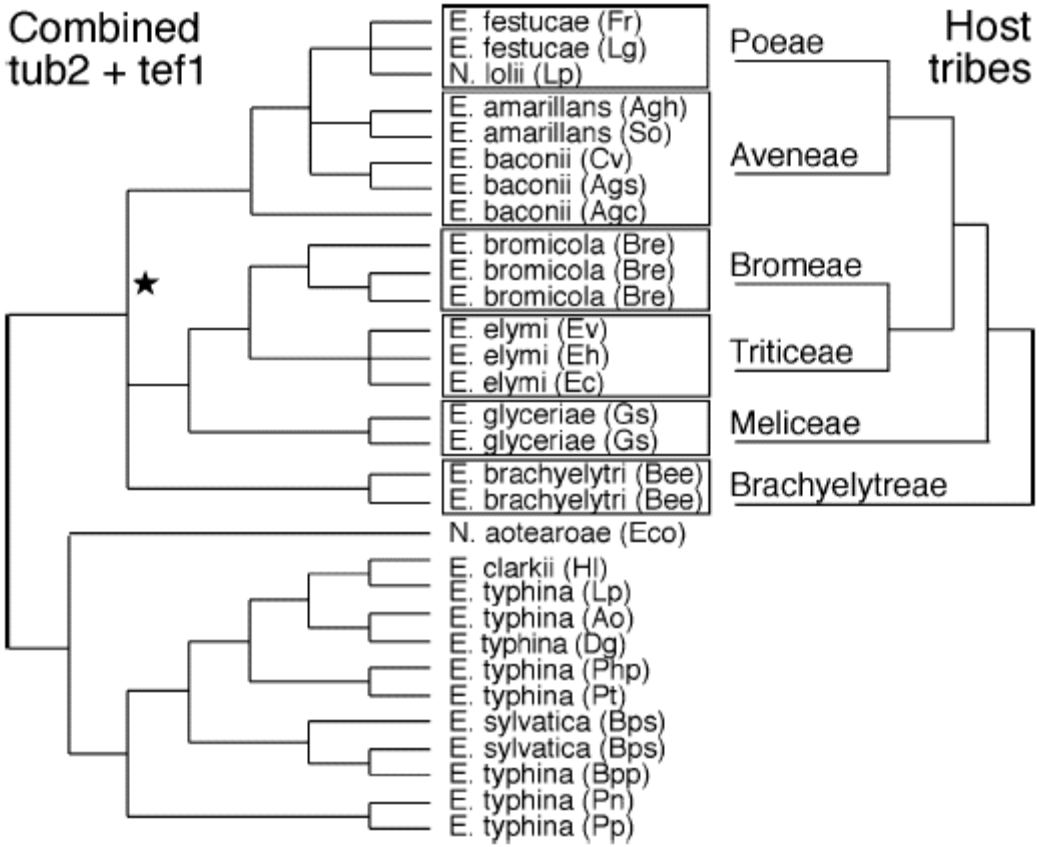
- *Epichloë* species have only one copy each of translation elongation factor α (*tef1*) and β-tubulin (*tub2*)
- Each gene gives similar phylogenetic relationship among taxa

- *N. uncinatum* has relationship with *E. bromicola* based on *tef1*, but with *E. typhina* based on *tub2*; suggests this species is a hybrid
- *N. coenophialum* has two copies of *tef1*, each having their own relationship to other taxa
- *N. coenophialum* has three copies of *tub2*, each having their own relationship to other taxa
- *N. coenophialum* thought to be the result of multiple hybridization events




Meadow fescue symbiotic with *Neotyphodium uncinatum*
Festuca glaucescens
Tall fescue ancestor with *Neotyphodium uncinatum*
Epichloë spore
First hybridization
Second hybridization
Tall fescue symbiotic with *Neotyphodium coenophialum*

Epichloë species and their grass hosts appear to have coevolved together



Neotyphodium-Tall Fescue Interactions: Economic, Agronomic, and Ecological Impact

Economic Impact

a.k.a. Impact on cattle and sheep

- Tall fescue is primary ground cover on 35 million acres (most in southeast); most important cool-season grass in U.S.
- Established pastures often have high levels of endophyte infection
- Endophyte produces a variety of secondary metabolites (mycotoxins)
- Ergot alkaloids lead to “fescue toxicosis”
  - Lower feed intake
  - Lower weight gains
  - Lower milk production
  - Higher respiration rates and body temperatures
  - Rough hair coats
  - More time spent in water and shade
  - Reduced reproductive performance
- **>$500 million annual loss**

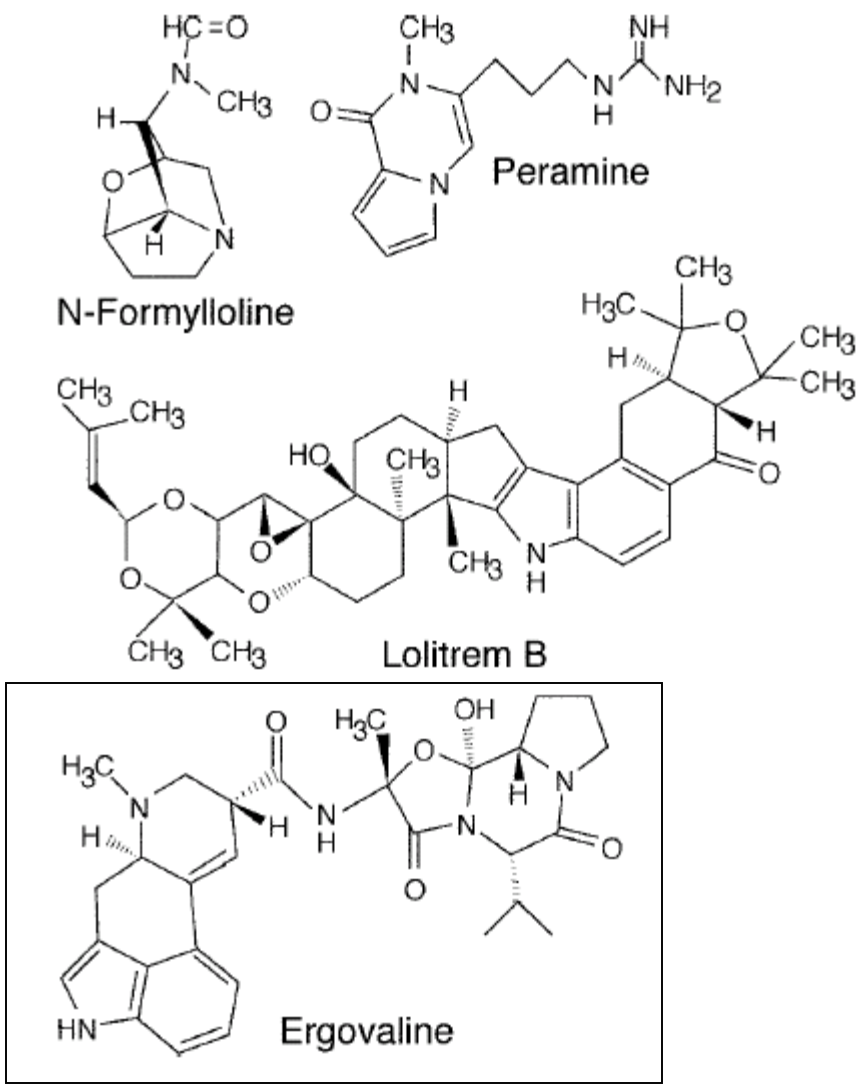
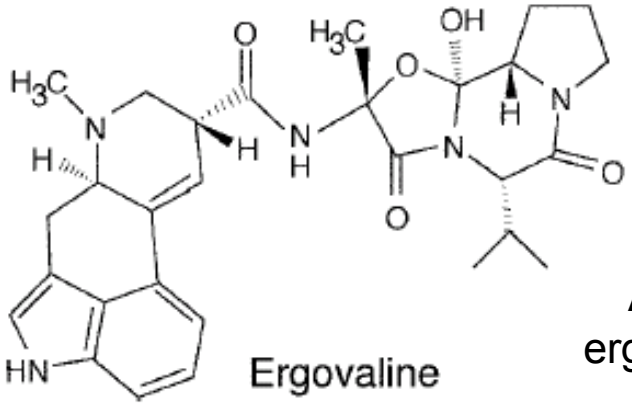


Figure 5: Examples of the four major classes of endophyte alkaloids. Lolitrem B, peramine, N-acetyl loline, and ergotamine represent indolediterpenoid, pyrrolopyrazine, aminopyrrolizidine and ergot alkaloids, respectively. Individual infected grasses may have various mixtures of alkaloids.

71

The Beggars (The Cripples)
1568

Pieter Bruegel The Elder



And other ergot alkaloids

***Claviceps purpurea* (a.k.a. ergot)**

Causes disease called “ergotism”, “St. Anthony’s fire”, or “holy fire”

Oldest recognized mycotoxicosis of humans (ergot alkaloids)

St. Anthony

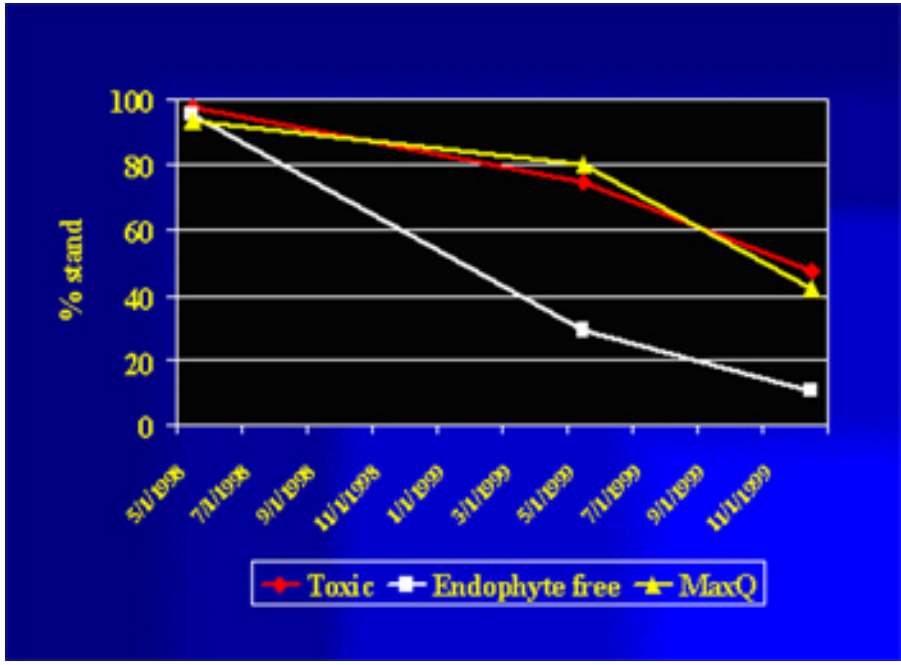
Protecting saint against fire, epilepsy, and infection

- Religious hermit in Egypt
- “Father of the monks”
- Died in A.D. 356 at age 105
- Crusade knights brought relics back to Dauphiné, France
- ~1090, an outbreak of “*ignis sacer*” (Holy Fire) in France
- Gaston, a wealthy citizen, and his son were among the afflicted
- Gaston prays to St. Anthony for a cure and promises to give all his wealth to aid other victims
- Order of Hospitallers of Saint Anthony

Agronomic/Ecological Impact

Infection is bad for cattle but great for the grass

- Endophyte infected tall fescue has……
  - greater stand persistence than does endophyte free fescue
  - increased resistance to insect herbivory
  - reduced herbivory from grazing livestock
  - increased resistance to plant pathogens
  - increased drought tolerance
  - increased production of tillers
  - increased photosynthetic rate
  - increased tolerance of nutrient limiting conditions



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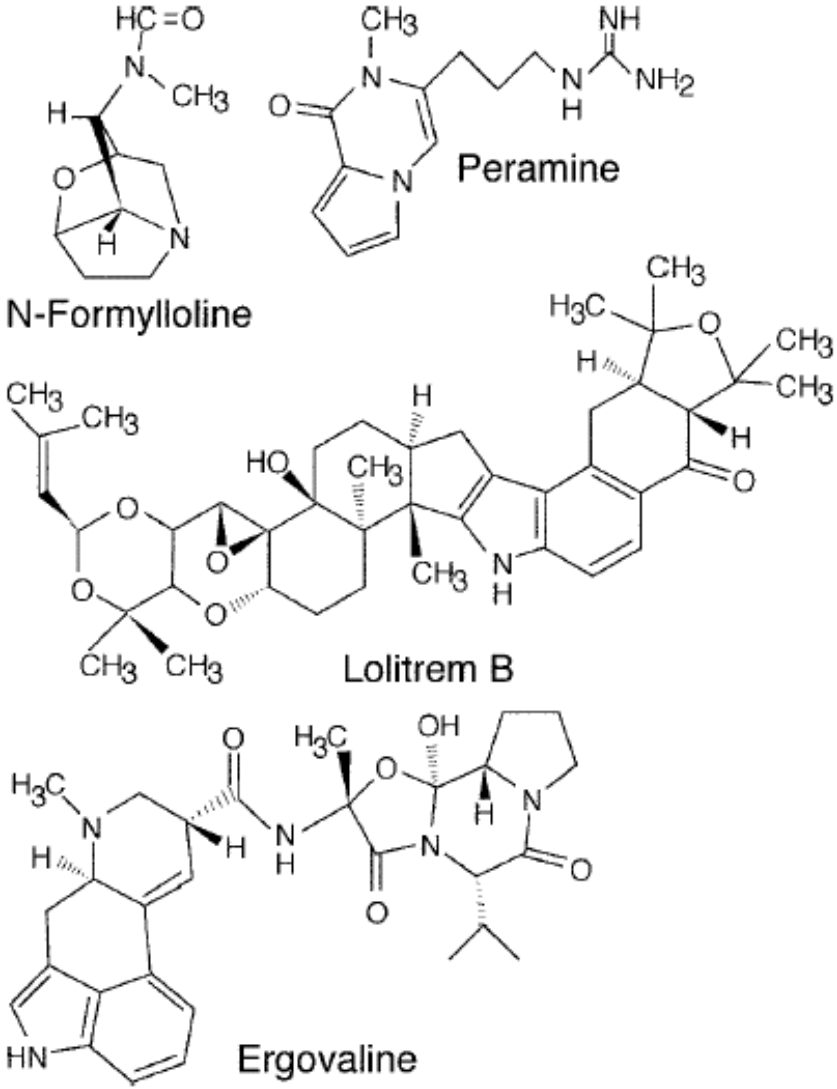
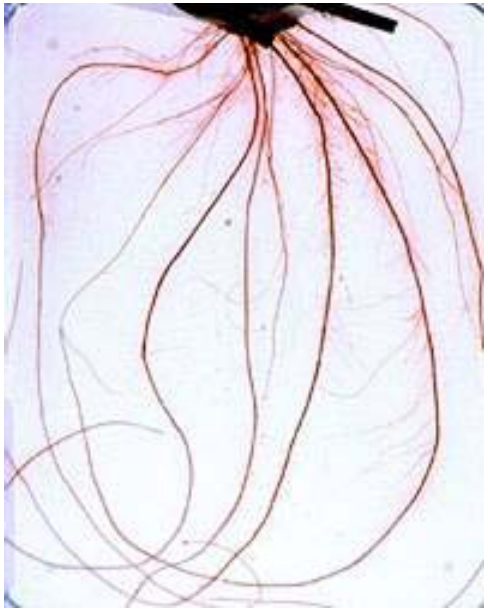


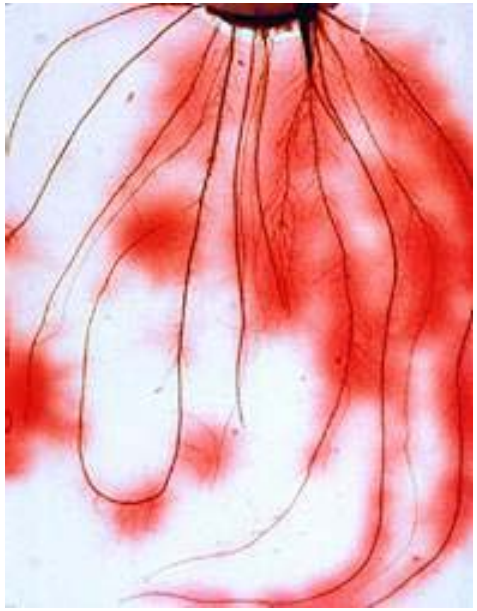
Endophyte-induced physiological changes to fescue roots

Noninfected and infected isolines grown under phosphorous deficient conditions

Endophyte-free



Endophyte-infected



- *Neotyphodium coenophialum* does not infect the roots of tall fescue.
- Yet the fungus stimulates roots to produce increased amounts of exuded phenolic compounds, as evidenced by the red staining.
- The compounds may have chelating ability, providing a larger pool of P.
- The compounds may also have a role in allelopathy and pathogen resistance

Ecological Impact (cont.)

Infection frequency of tall fescue increases over time

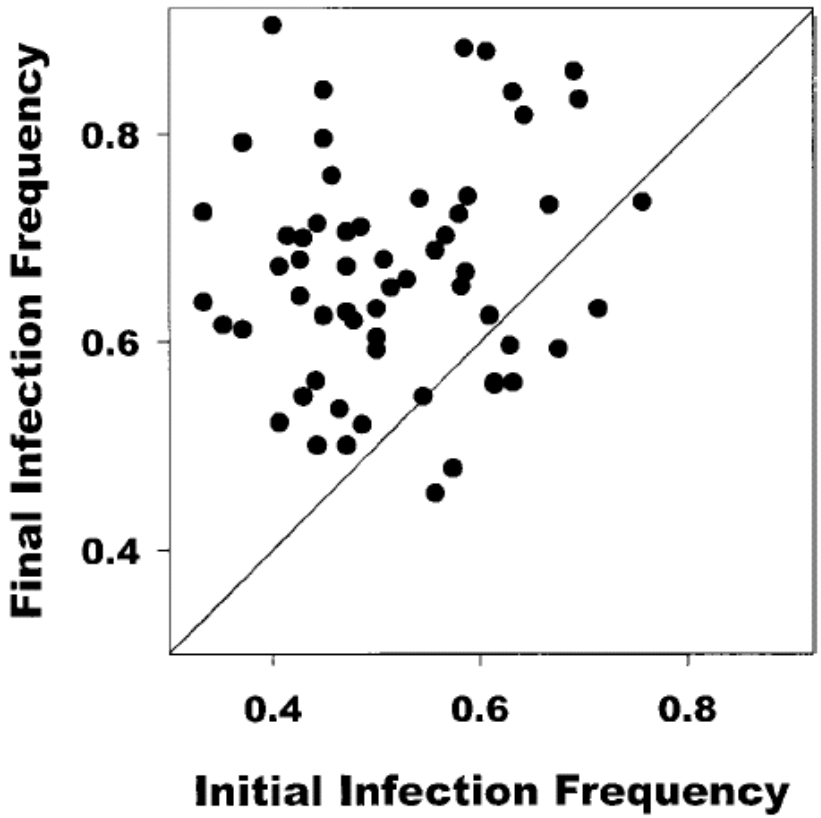
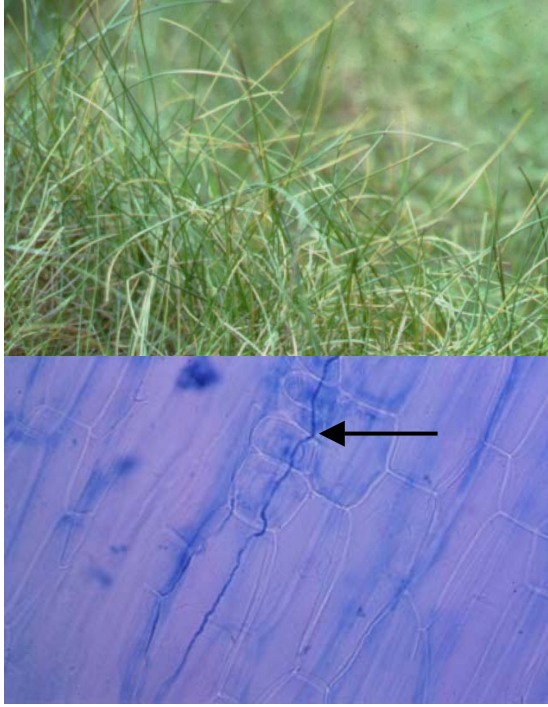


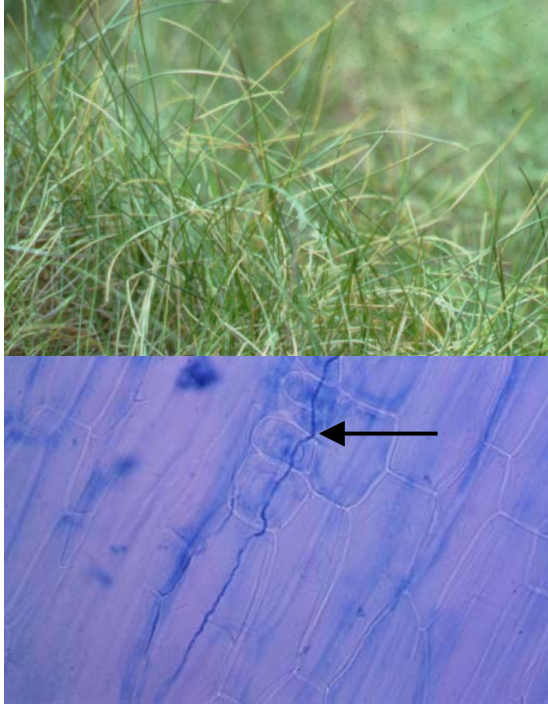
Figure 6: Change in infection frequency in tall fescue over a 4-yr period. Sixty plots were repeatedly sampled. Each point represents starting and final values for one plot. Diagonal line represents no change in infection frequency (K. Clay, unpublished data).

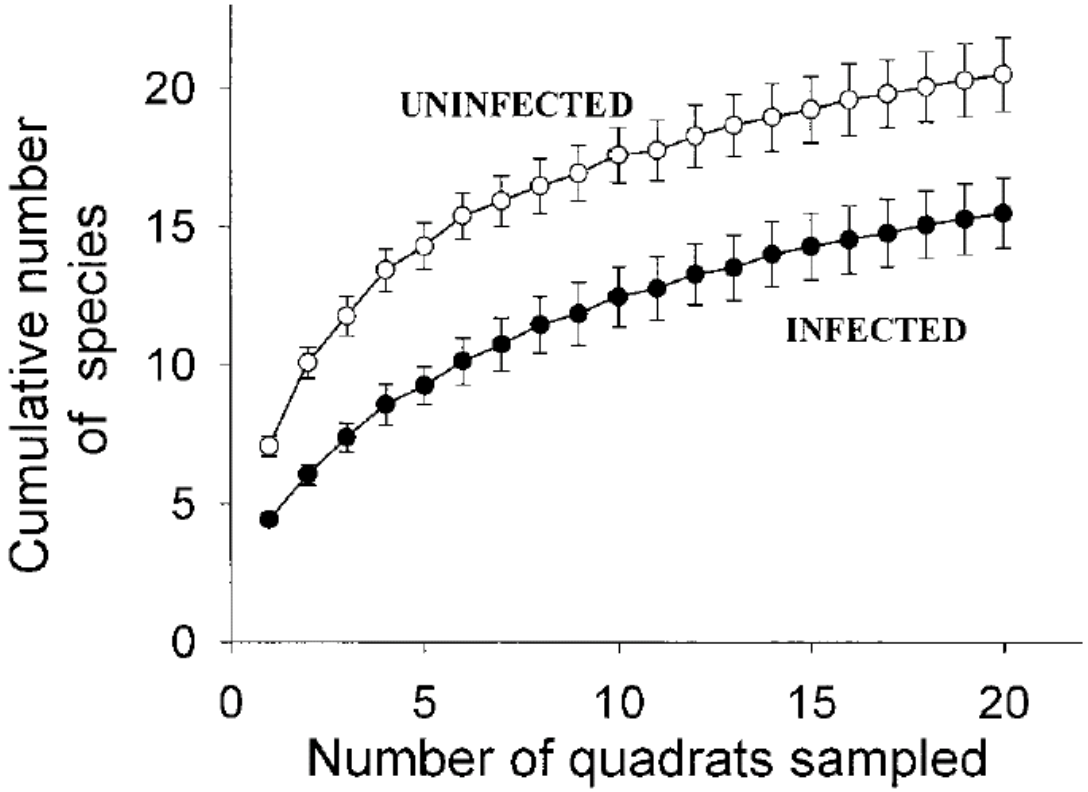


Remember, *N. coenophialum* is seed transmitted

Ecological Impact (cont.)

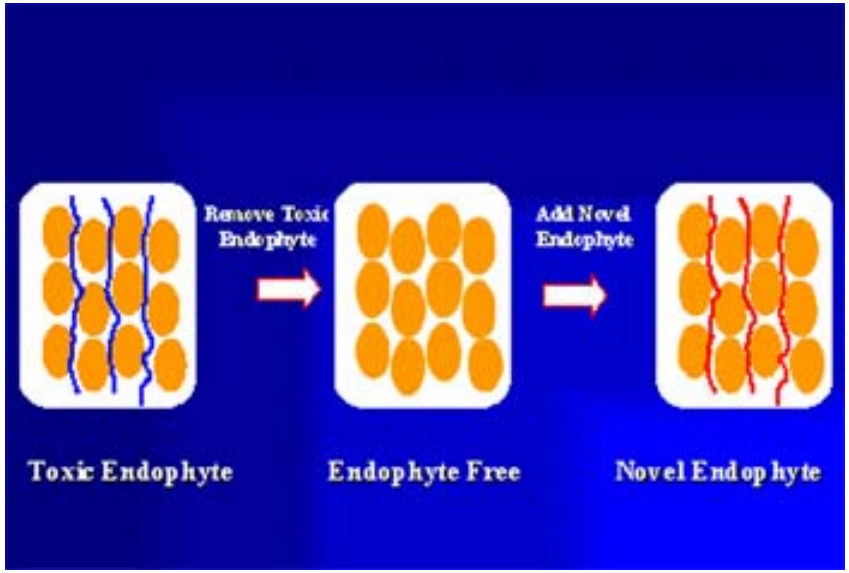
- Infected tall fescue significantly suppresses species diversity (other grasses and dicots)
- Uninfected plots undergo secondary succession more rapidly (more woody plants)
- Declines in populations of rabbit and other small mammal species attributed to increasing density of infected fescue





Generate non-toxic associations that retain positive characteristics

- Two approaches
  - Genetically engineer the native *N. coenophialum* to eliminate ergot alkaloids but retain the positive agronomic traits.
  - Create new (novel) associations using strains of *Neotyphodium* that do not produce ergot alkaloids. These strains were isolated from other grasses from other locations. This approach has lead to MaxQ, created by Joe Bouton here at UGA with collaborators in New Zealand.

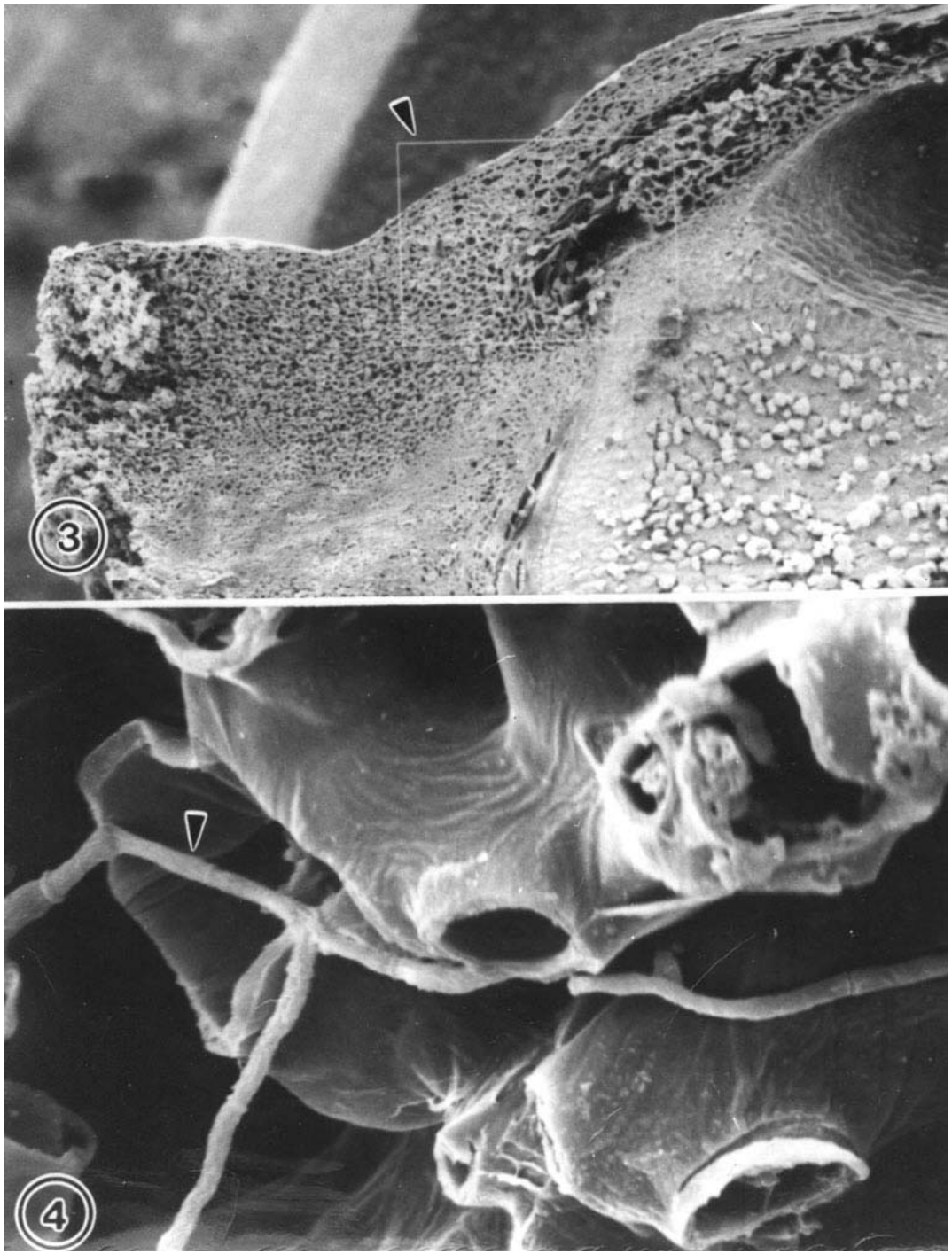


Fusarium verticillioides Interactions with Maize

❖ *Fusarium*-Corn Interactions

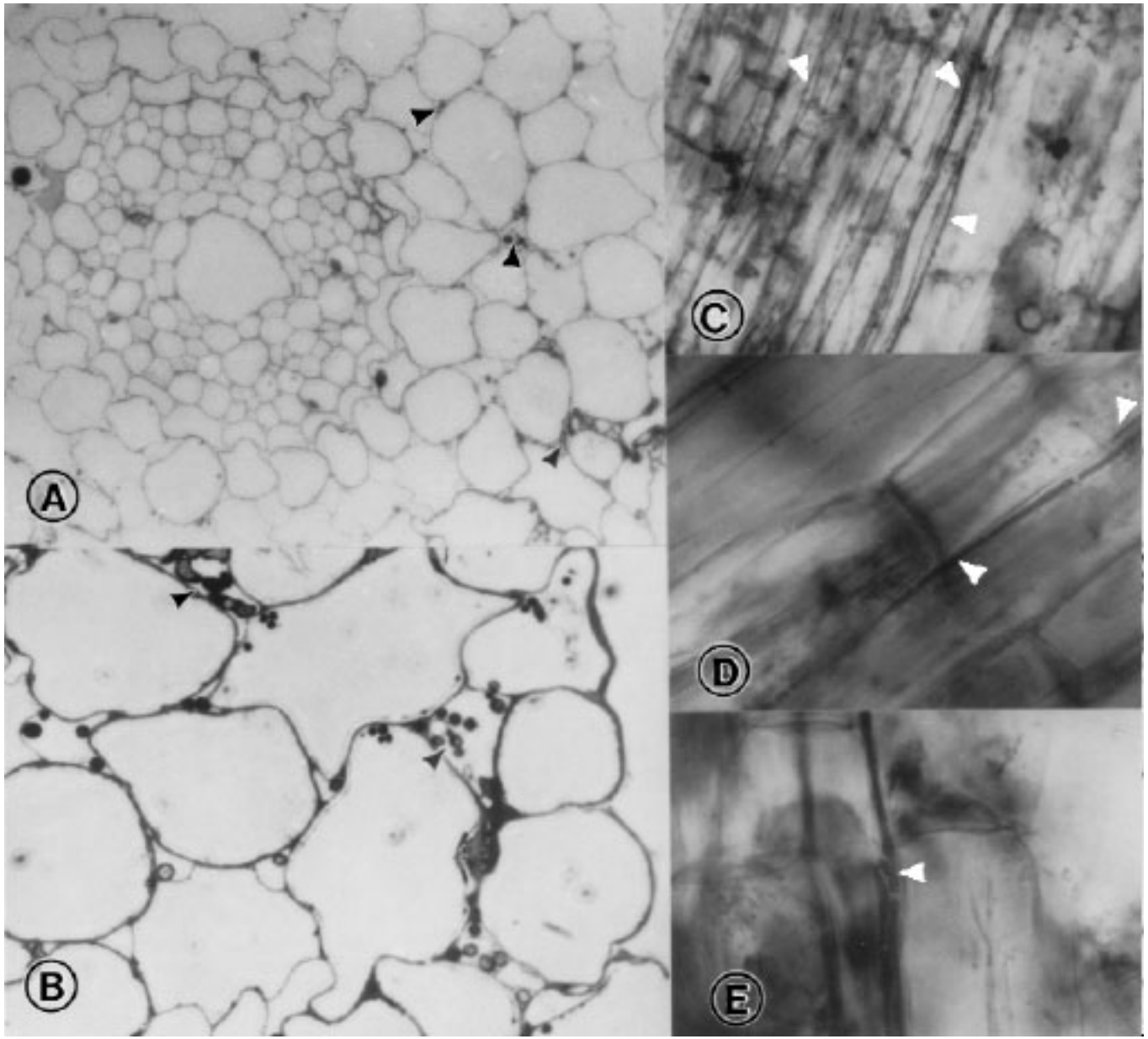
- *Fusarium verticillioides* is an endophyte of corn
- No obvious benefits to corn host, yet a ubiquitous association
- Systemic infections leading to non-embryonic infection of developing kernels
- >90% kernel infection has been reported
- Symptomless infections are common
- Diseased plants can result
- Ear rot is of greatest economic concern
- Contamination with fumonisin mycotoxins

Fusarium verticillioides
growing from asymptomatic corn kernel



Fusarium verticillioides infects the non-embryonic maternal tissue at the base of the kernel (the pedicel)

Fusarium verticillioides is endophytic within all maize tissues

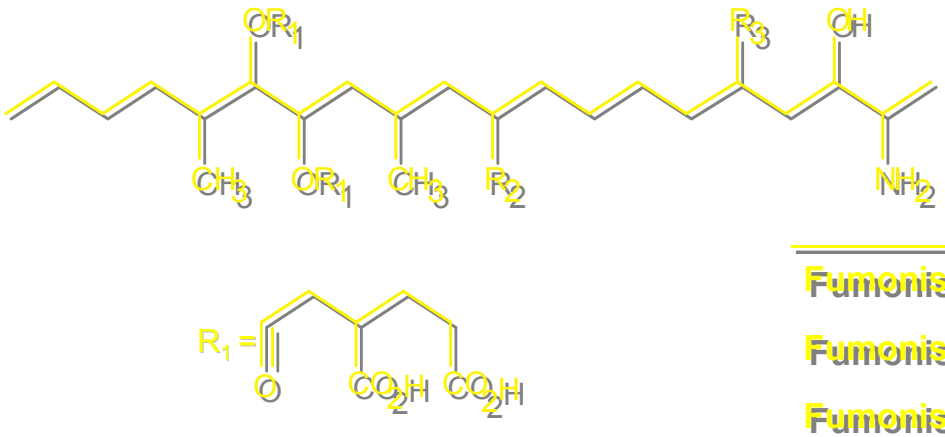


❖ *Fusarium* ear rot

- Often initiated by insect damage
- Results in reduced grain quality
- Leads to mycotoxin contamination
  - Fumonisin B_1
- Hazard to animal health

❖ Fumonisins

- Produced by *F. verticillioides* and related species; *in planta*
- Equine leucoencephalomalacia
- Porcine pulmonary edema
- Cancer in rats and mice.....FDA industry guidelines

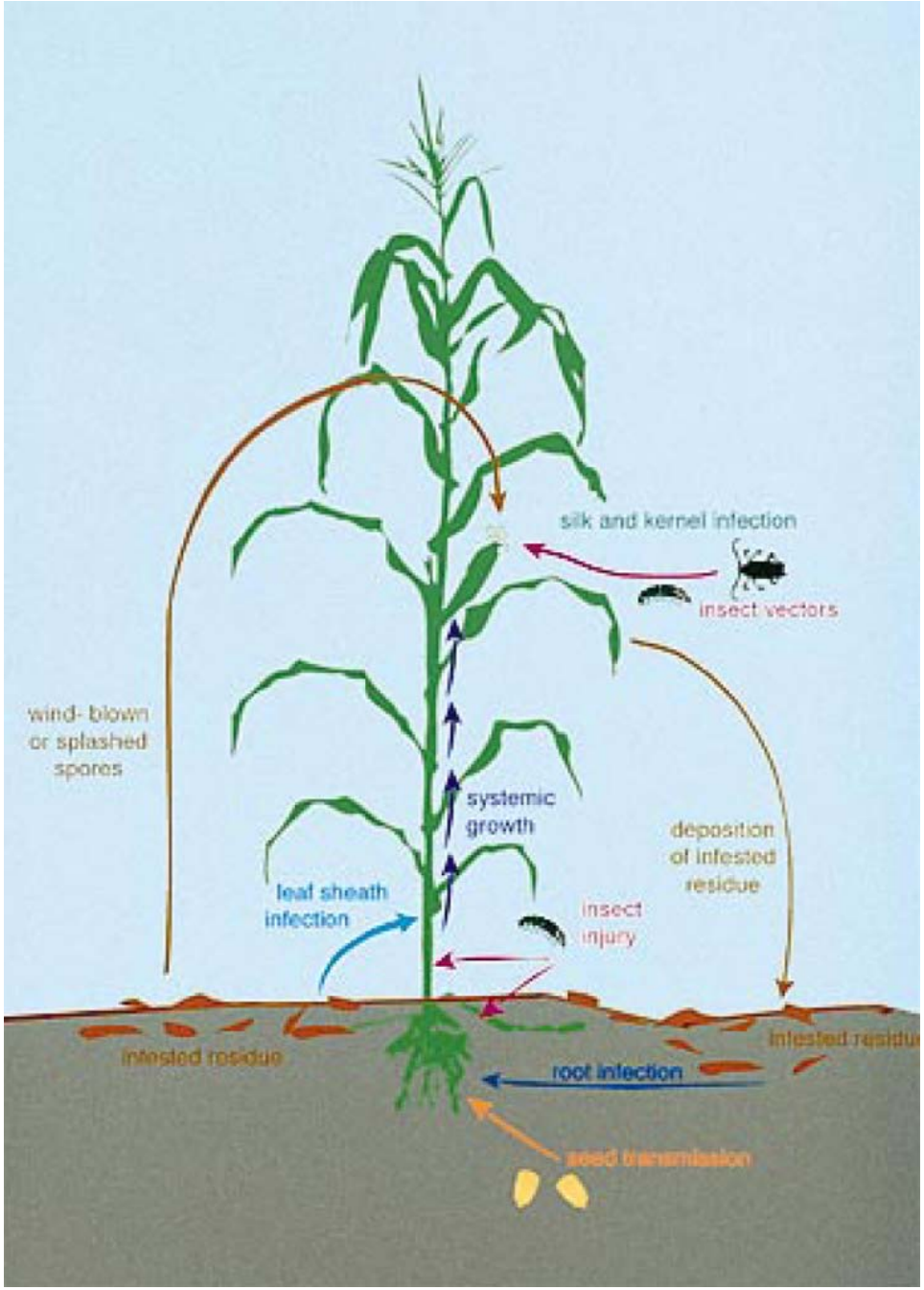


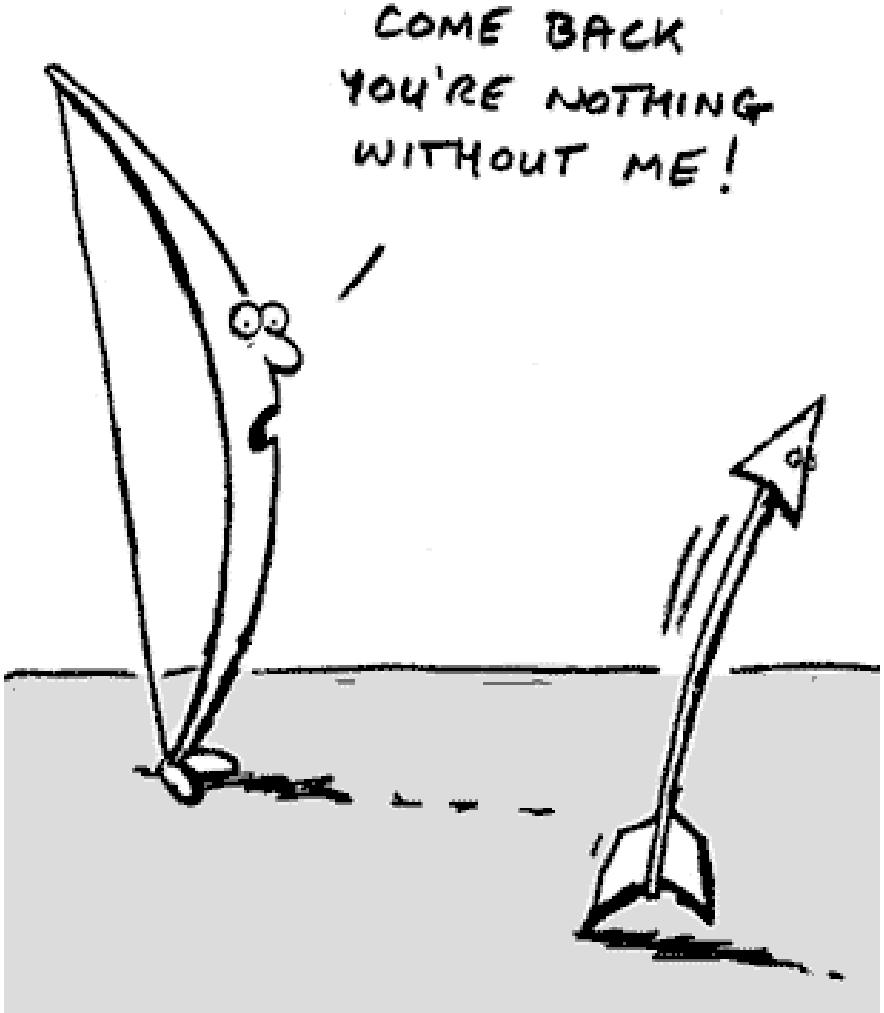
	R_2	R_3
Fumonisin B_1	OH	OH
Fumonisin B_2	H	OH
Fumonisin B_3	OH	H
Fumonisin B_4	H	H

Points of infection

Fusarium verticillioides can infect maize via seed transmission (vertical) as well as spore dispersal (horizontal)

Evidence suggests that most kernel infections occur via wind-blown spores that land on silks and grow down to the kernel




COME BACK
YOU'RE NOTHING
WITHOUT ME!