A Novel Inoculation Technique for Integrating the Endophyte *Epichloë festucae* into Perennial Ryegrass

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**Introduction**

The fungal endophyte *Epichloë festucae* var. *Lolii* is commonly associated with perennial ryegrass (*Lolium perenne*).

Some endophytes have been shown to reduce disease severity and often produce toxic alkaloids that can be harmful to mammals and invertebrates.

There is interest in incorporating novel endophytes into elite genotypic selections to improve cultivar disease resistance, reduce animal herbivory or replace an existing strain negatively impacting forage quality.

There are no methods papers reporting successful (>1%) integration of a novel strain into mature perennial ryegrass tissue (*Latch and Christensen, 1985; Simpson et al., 1997*). However, infecting sterile plant calli has proven successful (*Kaur et al., 2015*).

The objective of this study was to test a new inoculation technique that built upon previous methods, included different dark incubation periods and multiple endophyte strains (*Johnson-Cicalese et al., 2000; Wille et al., 1999*).

**Materials and Methods**

**Plant material**

- Four endophyte free (E-) genotypes of the perennial ryegrass variety ‘Spreader IV’ were selected.
- ‘Spreader IV’ is vigorous and has large pseudostems, making it easy to work with in the lab.
- Genotype selection was based off of ability to flower and produce seeds in the greenhouse with vernalization in the growth chamber.

**Endophyte strains**

- Endophytes were isolated from eight accessions of diverse origin.
- Isolations were conducted by sterilizing host pseudostem tissue and then incubating the tissue on potato dextrose agar for three weeks.
- Once hyphae were visibly growing from cut tissue ends, the endophyte was cultured and stored on fresh PDA.

**Endophyte detection**

- Endophyte infection was determined by both tissue print immunoblot and light microscopy.
- Tissue print immunoblot was done using a commercial kit (Agrinostics, Watkinsville, GA).
- Light microscopy was conducted by clearing sheath tissue with 95% EtOH, staining with rose bengal, and imaging under 400X magnification.

**Inoculation technique**

Endophyte free tillers were sterilized in 0.083% NaClO solution for 15 min. Loose or chlorotic sheath or leaf tissue was removed. Roots and leaves were trimmed to 1 cm.

Actively growing mycelium was chipped on a sterile petri plate in a flow hood. A sharp razor was used to cut pieces less than 1 mm wide.

A paste was made from the chopped mycelium using a scoopula. 1 ml of MS media (see below) was added to decrease the viscosity of the paste so it could be pressed through a hypodermic needle.

Mycelium paste was loaded into a 3 ml syringe with a 18 ga luer lock hypodermic needle. After running the paste through the 18 ga needle, the paste was loaded into a syringe with a smaller 22 ga needle.

Endophyte isolated from host tissue and cultured on PDA.

A 2-3 mm slit was made above the root tissue, running in a distal direction through the meristem. About 50 µL of paste was then injected into the slit made by the 22 ga needle. This step was conducted with a 4X lighted magnifying glass headset.

Injected tillers were placed into magenta boxes with 75 ml of 0.4% water agar (phytoblend), 1.11g/L Murashige and Skoog Basal Salts, pH adjusted to 5.8, and incubated for 18 days with 25 °C days and 15 °C nights. **Dark treatments** were applied for 2, 3, 4, 5 days in the chamber immediately following inoculation.

After 18 d the tillers were transplanted into sterile rockwool and covered with a plastic hood to increase humidity. After 7 d the tillers were transplanted into soilless media. Tillers were tested for the presence of endophyte 21 d post transplanting.

**Results and Discussion**

**Host survival**

Genotype survival was measured before testing for novel endophyte integration.

Results show that genotype 1 had the highest survival.

**Effect of endophyte strain**

50 days post inoculation, two strains had integrated into a novel host. Strain Y1 had an initial infection frequency of 25%.

80 days post inoculation all host tissue had lost infection.

**Effect of dark treatment post inoculation**

A longer dark period post inoculation increased infection frequency compared to shorter dark periods, however infection was lost over time.

**Conclusions and Future Directions**

- A novel inoculation technique was tested to introduce a non-native endophyte strain into a perennial ryegrass host.
- The four hosts that were tested had varying levels of survival.
- Two of the ten strains tested integrated themselves into the novel host for a short time.
- Inoculation in the dark increased the probability of endophyte integration.
- A formal experiment using only genotype 1 is underway that includes additional dark treatments and the *E. festucae* isolate “Rose City” along with several *E. lolii* isolates. We will also more carefully monitor the presence of the endophyte in host tissue over time.

**References**


