The seed of life – investigating *Epichloë* embryo colonisation

**Wei Zhang**1,2, **Stuart Card**2, **Craig McGill**1, **Wade Mace**2, **Michael Christensen**2, **Cory Matthew**1

1Institute of Agriculture and Environment, Massey University, Palmerston North, New Zealand
2Forage Improvement, AgResearch Ltd., Grasslands Research Centre, Palmerston North, New Zealand

*Retired Scientist*

w.zhang1@massey.ac.nz

**INTRODUCTION**

Asexual cool-season grass endophytes of the genus *Epichloë* are strictly vertically disseminated via host seed. During the plant's sexual reproductive cycle, these fungi colonise the floret through the base of the ovary and subsequently develop in the nucellus tissue that surrounds the embryo sac. At seed maturity, the endophyte is distributed primarily in the embryo and in-between the aleurone and pericarp layers. However, there is conflicting evidence on the timing of embryo colonisation.

**AIM**

Determine when *Epichloë* endophyte hyphae enter the host grass embryo.

**RESULTS & DISCUSSION**

Endophyte hyphae were observed in all stages of seed development from Stage I to Stage V. The *Epichloë* hyphae were easily observed in all flower and seed tissues including the early stages before fertilisation (Figure 2A). At Stage II, the ovary length had increased and the embryo sac vacuole enlarged where hyphae were observed attached to the large antipodal cells (Figure 2B). Later at Stage III, hyphae could be seen in-between the young embryo cells at the base of the ovary near the micropyte (Figure 2C).

With the onset of seed filling, the nucellus layer was crushed by the developing endosperm (es) (Figure 3A, B, C). Cross sections of ovaries at Stage III and Stage IV (Figure 1) showed that endophyte hyphae were found among the pericarp cells, the vascular bundles outside the ovule (vb), the nuclear projection (np), and the outer and inner integuments (oi, ii) (Figure 3E, F). From Stage II to Stage IV, more endosperm started to accumulate inside the embryo sac where endophyte hyphae was observed in-between the non-starchy endosperm cells (Figure 3F).

Fluorescent staining indicated that viable endophyte hyphae were present in the ‘infection layer’ and embryo axis in mature seed (Figure 4A, B, C).

**CONCLUSION**

Host seed colonisation is an essential part of the *Epichloë* lifecycle and very few researchers have investigated this area in detail. We show, using fluorescent confocal microscopy, that endophyte hyphae enter the flower tissues at a very early stage and are associated with the embryo sac before fertilisation takes place, and later in the embryo axis in the mature seed.

**REFERENCES**


**ACKNOWLEDGEMENTS**

We thank Natasia Forester and Brian Tapper for technical assistance, and Rachel Hunt for poster design input. We thank Grasslands Technology Ltd., PGS Wrightson Seeds Ltd. and China Scholarship Council for financial support.
The seed of life, investigating Epichloë endophyte colonisation.

Zhang, W

2015