

Mechanical stress initiates intercalary growth in *Epichloë* fungal symbionts of grasses.

K. G. Sameera U. Ariyawansa¹, Rosie E. Bradshaw², Neil A.R. Gow³, Nick D. Read⁴, Richard D. Johnson¹, Duane P. Harland⁵, Christine R. Voisey¹.

1) Plant Fungal Interactions, AgResearch Grasslands, Palmerston North, Manawatu, New Zealand; 2) Institute of Fundamental Studies, Massey University, Palmerston North, New Zealand; 3) School of Medical Sciences, University of Aberdeen, United Kingdom; 4) Manchester Fungal Infection Group, University of Manchester, United Kingdom; 5) AgResearch, Lincoln Research Centre, Christchurch, New Zealand.

Colonization of aerial grass tissues by seed-transmitted *Epichloë* endo-symbionts initially occurs through ramification of hyphal tips between cells of the host shoot apical meristem (SAM). Uniquely, when hyphae in the SAM start to invade developing leaves, growth ceases at apices, and hyphae extend via intercalary growth (division and extension in non-apical compartments). We hypothesise that intercalary growth is stimulated by mechanical stretch imposed on hyphae by their attachment to elongating host cells, and that this stress is sensed by mechano-sensors located on hyphal membranes. Deletion of *E. festucae* *mid1*, a putative orthologue of the *mid1* yeast mechanosensor, and a component of the Mid1/Cch1 calcium channel, reduced *E. festucae* radial growth rate in culture, caused aberrations in hyphal cell walls, and greatly restricted intercalary growth in infected plants. A technique to mimic the hyphal stretching proposed to occur in planta has been developed and tested on wild type *E. festucae* growing in culture. Intercalary compartments remained viable despite being stretched to 20% of their original length, and stretching also initiated de novo mitosis and septation in intercalary compartments. Calcium imaging experiments on *E. festucae* growing in culture have revealed that the Mid1 protein is responsible for calcium pulses at the hyphal tip during growth, and that the calcium originates from the exterior of the hypha and not from calcium stores. Studies are underway to characterise calcium signalling in intercalary compartments in wild type and the Δ *mid1* deletion mutant when subjected to mechanical stress.