DISTRIBUTION OF *NEOTYPHODIUM LOLII*-ENDOPHYTE METABOLIC ACTIVITY IN PERENNIAL RYEGRASS (*LOLIUM PERENNE*, L.) AND ITS IMPLICATIONS FOR ALKALOID DISTRIBUTION AND PHOTOSYNTHESIS

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Abstract

Neotyphodium lolii is a fungal endophyte of perennial ryegrass (*Lolium perenne*). In this symbiosis, alkaloids are produced that significantly impact on the performance of farmed animals. Little was previously known about the physiological conditions for the endophyte in the plant leading to their production. A strain of *N. lolii,* previously transformed with the ß-D-glucuronidase (GUS) gene of *E. coli* under the control of a constitutive fungal promoter, was used for investigations into the *in planta* metabolic activity of the endophyte; distribution of the alkaloids ergovaline, peramine, and lolitrem B; and photosynthesis.

In vitro studies with this transformed *N. lolii* strain demonstrated the utility of constitutive GUS expression for assessing the metabolic state of the endophyte. By using constitutive GUS expression and a method for quantitation of the *in planta* biomass of the endophyte, the endophyte metabolic state (EMS) in the grass plant was determined. The EMS was high and uniform in plant tissues and genotypes differing in endophyte concentration, indicating that proliferation of the endophyte in the plant is not controlled *via* the EMS.

Ergovaline, peramine, and lolitrem B exhibited each a characteristic within-tiller distribution maintained across different plant genotypes. None of the alkaloids was distributed in exact proportion to the distribution of metabolically active endophyte mycelium. Differences in the accumulation of the alkaloids per mycelium were observed between plant tissues and plant genotypes, suggesting differential rates of synthesis and/or degradation of the alkaloids in the mycelium and translocation within the grass tiller.

Rates of net photosynthesis at high light intensities were lower in plants infected by *N. lolii*, indicating for an effect on photosynthetic capacity. However this effect was plant-age dependent. Plant growth was not strongly affected by the endophyte, but infected plants had consistently lower leaf elongation rates. The changes in leaf elongation and photosynthetic capacity in infected plants might signal for an effect of *N. lolii* on the nitrogen metabolism of its host.

The experiments concerning the *in planta* EMS, alkaloid distribution, plant growth, and photosynthesis were conducted in a controlled environment the establishment of which was part of this study. In addition, for the detailed mapping of ergovaline within the grass tiller a method for quantitative extraction of this alkaloid was developed and optimised.

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Related Publications

Some of the material presented in this thesis has been published:

Schmid, J., Spiering, M. J. & Christensen, M. J. (2000). Metabolic activity, Distribution, and Propagation of Grass Endophytes *in Planta*: Investigations Using the GUS Reporter System. In *Microbial Endophytes* (White, J. F. W., Jr. & Bacon, C. W., eds.), pp. 295-322. Marcel Dekker, New York.