Metabolome Analysis of the Interaction Between Perennial Ryegrass (*Lolium Perenne*) and the Fungal Endophyte *Neotyphodium Lolii*

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Introduction Perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) frequently contain endophytic fungi (*Neotyphodium lolii* in perennial ryegrass and *N. coenophialum* in tall fescue). The presence of the endophyte has been shown to improve seedling vigour, persistence and drought tolerance in marginal environments as well as provide protection against some insect pests. Endophyte-infected grasses also produce a wide range of metabolites, including ergopeptine alkaloids, indole-isoprenoid lolitrems, pyrrolizidine alkaloids, and pyrrolopyrazine alkaloids. In contrast to information on alkaloids and animal toxicosis, the beneficial physiological aspects of the endophyte/grass interactions have not been well characterised. The physiological mechanisms which lead to increased plant vigour and enhanced tolerance to abiotic stresses unrelated to the reduction in pest damage to endophyte-infected grasses are unknown. Recent technological advances in metabolomics enable dynamic changes in the metabolome of an organism under varying experimental conditions to be studied. This provides opportunities for the investigation and validation of each and every detected metabolite, investigation of known metabolic pathways through searching of databases of known metabolites, molecular formula determination of unknown metabolites and creation of pathways from novel metabolites.

Materials and methods Endophyte-infected (E+) and endophyte-free (E-) ryegrass plants in an isogenic host genetic background were generated, characterised by SSR markers and ELISA and used for metabolic fingerprinting and metabolic profiling.

Results Within the context of a spatial and temporal systems biology approach, a metabolomics study of the mutualistic interaction between perennial ryegrass and its fungal endophyte was undertaken. Polar and lipophilic compounds were extracted from endophyte, E+ ryegrass and E- ryegrass standardised samples, and analysed (fractionation and mass determination) by gas chromatography (GC)/mass spectrometry (MS) and high performance liquid chromatography (HPLC) with photodiode array detection (PDA)/mass spectrometry using Q-TOF II MS with CapLC, Q-TOF Ultima API MS with Alliance HT and PDA, and GC-TOF Leco/Pegasus III MS with CTC PAL sampler and Agilent GC injector. Mass profile data were processed using MetAlign™ software and subjected to PCA. Mass peaks showing significant changes in comparative assessments between E+ ryegrass and E- ryegrass samples allowed the identification of sample-specific and differentially accumulating compounds (Figure 1), indicating specific biochemical pathways that may lead to enhanced tolerance to abiotic stress in the endophyte-grass host interaction.

			neg	pos	
scan	rt min	mass	mean	mean	fold
125	2.38	881	21	45	2.2
432	8.20	248	17	92	5.6
698	13.27	562	18	545	30.9
698	13.27	563	18	481	27.2
716	13.61	562	18	551	31.2
717	13.63	563	18	483	27.3
911	17.33	147	31	73	2.3
911	17.33	361	68	139	2.0
1013	19.27	701	22	46	2.1
1025	19.49	554	18	465	26.6
1025	19.49	555	18	411	23.0
1027	19.54	440	18	142	7.9
1028	19.56	562	18	1080	61.1
1028	19.56	563	18	944	53.4
1029	19.58	564	17	104	6.2
1284	24.43	441	17	110	6.5
1478	28.14	219	20	41	2.1
1936	36.88	835	17	55	3.1
2222	42.30	827	18	53	2.9
2248	42.79	626	18	45	2.6
2333	44.42	701	84	203	2.4

Figure 1 Analysis of mass profile data from metabolic fingerprinting/profiling of the association between perennial ryegrass and *Neotyphodium lolii*