Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 68 (2007) 355–360
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Peramine and other fungal alkaloids are exuded in the guttation fluid of endophyte-infected grasses

Albert Koulman *, Geoffrey A. Lane, Mike J. Christensen, Karl Fraser, Brian A. Tapper

AgResearch Grasslands Research Centre, Forage Biotechnology, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand

Received 1 August 2006; received in revised form 12 October 2006
Available online 28 November 2006

Abstract

Many grasses live in association with asymptomatic fungi (*Neotyphodium* spp. endophytes), which grow in the intercellular spaces of the grass. These endophytes produce a range of alkaloids that protect the grass against grazing by mammals and insects. One of these alkaloids is an unusual pyrrolopyrazine, peramine. Peramine appears to be continuously produced by the endophyte, but does not progressively accumulate. No mechanism for the removal of peramine by its further metabolism or any other process has been reported. Our aim was to detect peramine or peramine metabolites in plant fluids to determine if peramine is mobilized, metabolized or excreted by the plant. We also wanted to determine if other fungal metabolites are mobilized by the plant, as has been proposed for the loline alkaloids.

We developed a highly sensitive method for the analysis of peramine, using a linear ion trap mass spectrometer. We studied the fragmentation pathway of peramine using ESI MS^n and ESI FTICRMS. Based on these results we developed a single reaction monitoring method using the fragmentation of the guanidinium moiety. Cut leaf fluid and guttation fluid of different grass endophyte associations (*Lolium perenne* with *Neotyphodium lolii*, *Festuca arundinacea* with *Neotyphodium coenophialum*, and *Elymus* sp. with *Epichloë* sp.) were analysed. Peramine was detected in the cut leaf fluid of all grass-endophyte associations, but not in the guttation fluid of all associations. In some associations we also detected lolines and ergot peptide alkaloids. This is the first report showing the mobilization of fungal alkaloids into plant fluids by the host plant in grass-endophyte associations.

Keywords: *Lolium perenne*; *Neotyphodium lolii*; Poaceae; Guttation; Alkaloid mobilization; Ergot alkaloids; Lolines; Ergovaline; Peramine; LCMSMS

1. Introduction

Perennial Ryegrass (*Lolium perenne* L.) is the most important pasture grass species in New Zealand. This plant commonly grows in association with a fungal endophyte *Neotyphodium lolii* (Latch, Christensen, & Samuels) Glenn, Bacon & Hanlin that lives in the intercellular spaces of the grass. The presence of the endophyte significantly improves the agricultural value of the plant, as it makes it less vulnerable to pests (Breen, 1994; Bush et al., 1997; Popay and Bonos, 2005). However, endophyte presence can also severely impair the performance of grazing livestock. Both of these effects derive primarily from the range of alkaloids produced by the endophyte within the grass host. Some classes of alkaloids, such as ergot alkaloids and indole diterpenes, cause animal toxicoses such as ryegrass staggers (Siegel et al., 1985; Fletcher, 2005). Other alkaloids produced by the endophyte, like the unusual pyrrolopyrazine alkaloid peramine, deter herbivorous insects (Rowan and Gaynor, 1986).

The presence of the endophyte in the plant does not give rise to any noticeable morphological phenotype and it appears that there is balanced metabolic interaction between the plant and the endophyte. We have a very limited understanding of this interaction. A recent study showed that each of the classes of alkaloids has its own characteristic intra-plant distribution in ryegrass, although the distribution is variable between the different host genotypes (Spiering et al., 2005). The main indolediterpenoid,

* Corresponding author. Tel.: +64 6 356 8019; fax: +64 6 351 8032.
E-mail address: albert.koulman@agresearch.co.nz (A. Koulman).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.10.012

lolitrem B, accumulates over time in older tissue and is present at only low levels in young tissue. Ergovaline is concentrated in the stem and basal leaf sheath of intermediate age. Peramine, however, is fairly evenly distributed in plant tissues and does not accumulate in older tissues (Ball et al., 1997; Spiering et al., 2002, 2005). One of the explanations for these differences in distribution could be that lolitrem B remains within the endophyte hyphae, and ergovaline is associated with fungal growth in particular tissues, while peramine is translocated from the endophyte into plant intercellular spaces where it is either metabolized or mobilized.

One way in which plants are able to dispose of unwanted compounds is via the mechanism of guttation, a process occuring under conditions of high humidity in which excess water is exuded. The guttation droplets are formed by special tissues called hydathodes, which are located at the tip of the leaf (Taiz and Zeiger, 1991). It has been shown that xenobiotics, like griseofulvin and other antibiotics, which are taken up by the roots and are not metabolizable, can be excreted into the guttation fluid (Bollard, 1960). Because peramine is not produced by the plant itself, our hypothesis was that peramine released by the endophyte into the apoplastic spaces could appear in guttation fluid. We expected the levels of peramine to be low and therefore difficult to measure with existing HPLC-UV analysis (Spiering et al., 2005). We developed a LCMSMS method with increased sensitivity to peramine plus capability to detect possible metabolites.

2. Results and discussion

To reach sufficient sensitivity for the analysis of peramine, we developed a LCMSMS method based on the fragmentation of the guanidinium part of the peramine molecule. The fragmentation pattern of peramine under collision-induced dissociation was studied using ion trap mass spectrometry, as an enhanced understanding of this fragmentation mechanism would aid identification of possible metabolites of peramine. Hypothetical fragment formulae were validated with accurate mass data obtained through ESI Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS). The main fragments (see Fig. 1) of peramine derive from the degradation of the guanidinium moiety, while the pyrrolopyrazine skeleton appears to be much more stable. The main fragment has a m/z of 206 (exact m/z 206.12877 $C_{11}H_{16}O_1N_3$) corresponding to loss of diazomethane (CN_2H_2), which has been observed for similar dipeptide products (Fabricant et al., 2005). A synthetically prepared analogue of peramine (homoperamine) showed the same fragmentation pattern (data not shown). This ion undergoes further fragmentation to two distinct ions with m/z of 175 and 149, which are also present as minor ions in the mass spectrum of peramine itself. The other main ion in the spectrum of m/z 231 is the product of loss of ammonia (NH_3). Further

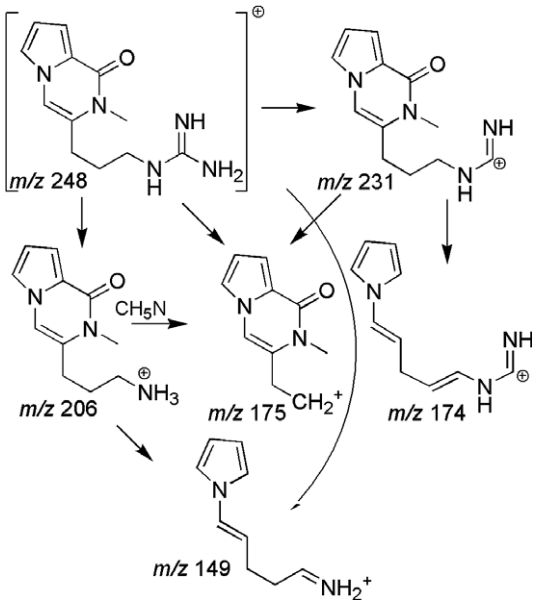

Fig. 1. Fragmentation of peramine (m/z 248) in linear ion trap using 25% relative collision energy.

fragmentation of this ion does not yield the 206 ion, which is additional evidence for the diazomethane loss of peramine. Because the diazomethane loss is rare for natural products we decided to use this for single reaction monitoring for the quantitation of peramine and used it as well to search for possible metabolites of peramine.

Chromatography on a reverse-phase column coupled with fragmentation from m/z 248 to 206, yields both quantitative and qualitative information in the analysis of peramine with high sensitivity. The MSMS detection of peramine is roughly 50-fold more sensitive than UV detection: the limit of detection using 290 nm with UV was 40 ng ml^{-1}, while with the SRM detection the limit was 0.8 ng ml^{-1}. Calibration curves showed sufficient linearity and precision (coefficient of variance between 5 and 10% in the different experiments) for routine use over a range of 10 μg ml^{-1} to 5 ng ml^{-1} (LOQ was 5 ng ml^{-1}). The control samples showed an average variance of 6%. The mass spectrometer was additionally operated in a data-dependent selection mode, to enable detection of possible metabolites of peramine or other alkaloids from the endophyte.

Droplets were collected from intact leaf tips and cut leaves from several grass endophyte associations. These included the important naturally-occurring forage grass endophyte associations, perennial ryegrass plant (*Lolium perenne*) "Nui 5" with *Neotyphodium lolli* strain AR93, and tall fescue (*Festuca arundinacea*) plants with *Neotyphodium coenophialum*, and a synthetic association of perennial ryegrass plant G1057 with *Epichloë festucae* strain Fl1. Additionally, we selected two naturally occurring *Elymus*/endophyte associations found to have a marked difference in peramine production. These are associations from different seed accessions originating in China, seedlines BZ2153 (low peramine, "Elymus 1") and BZ2155 (high peramine,

"Elymus 2"). The plants were identified as being different *Elymus* spp. by the authors. The associated endophytes of these two associations have been classified as *Epichloë* spp. Stromata characteristic of this sexual genus were formed on several of the reproductive tillers of the "Elymus 1" clonal plants. No stromata were produced on reproductive tillers of the clonal plants of "Elymus 2", however, the colony characteristics of this endophyte were very similar to those of the *Epichloë* endophyte from "Elymus 1" including the presence of conidia ~5 microns long, indicating that it is also an *Epichloë* sp.

Peramine was detected in the cut leaf fluid from all studied associations (see Table 1). In the fluid we did not find any mass spectroscopic evidence for the presence of any metabolite of peramine, and no other peaks in the chromatograms correlated with the presence of peramine. The only other compound detected in the fluid which showed a guanidinium group loss in the mass spectrum was arginine, also observed in the endophyte-free material. The levels of peramine in both cut leaf fluid and in guttation fluid differ quite widely from plant to plant (see standard deviations in Table 1). The levels of alkaloids produced by different grass-endophyte associations have previously been observed to differ appreciably (Keogh et al., 1996; Ball et al., 1997; Spiering et al., 2002) and even between clonal plants with the same endophyte (Spiering et al., 2005). In addition differences in root pressure may cause even more fluctuations in the final concentration of peramine in the fluids. The presence of peramine in guttation fluids shows unambiguously that peramine is mobilized from within the fungus, taken up into the xylem and exuded via the hydathodes. While mobilization of endophyte alkaloids within the plant has been previously postulated (e.g. Spiering et al., 2005), this is the first study providing direct evidence that alkaloids produced by the endophyte are mobilized and translocated by the host plant.

Peramine was found in the guttation fluid of four out of the five associations studied (see Table 1). Under humid conditions plants are able exude water through the leaf tips, a phenomenon known as guttation. Because valuable compounds could be lost through this process the plant has the ability to re-uptake valuable compounds through different transporter proteins, like the re-uptake of cytokines by PUP (Bürkle et al., 2003). In contrast under some circumstances plants are able to exude superfluous amounts of their own metabolites. Thus Arabidopsis plants overexpressing the protein glutamine dumper1 exude high amounts of glutamine into the guttation fluid leading to crystallization (Pilot et al., 2004). It is not clear to what extent plants regulate their internal levels of compounds through guttation. Plants are also able to dispose of unwanted compounds through this mechanism, like xenobiotics (as mentioned above). Imidacloprid, a systemic insecticide, was routinely used to control glasshouse pests like the mealybug in the green-house and this compound and its main metabolite (4-hydroxy-imidacloprid) were detected in both cut leaf fluid and guttation fluid by LCMSMS. The mass spectra of both compounds were in correspondence with the published data (Laurent and Rathahao, 2003).

Across the different associations studied there was no consistent relationship between the levels of peramine detected in fluid from cut leaves and in guttation fluid. Tall fescue apparently retains peramine inside the plant, while for perennial ryegrass the concentration of peramine in exuded fluid was at a similar levels to that inside the leaf. By contrast the concentration of imidacloprid and its metabolite was up to ten-fold higher in guttation fluid than in cut-leaf fluid, suggesting the deliberate exudation of these compounds. There appear to be systems available to either actively exude (Pilot et al., 2004) or reuptake metabolites (Bürkle et al., 2003), but whether both systems are available in one species is not yet clear. The marked differences in exudation patterns observed here suggest the plant is able to regulate the process, and that there is specific exudation of peramine in perennial ryegrass or a specific retention by tall fescue. At the moment the process of guttation fluid formation is not well enough understood to choose between these mechanisms.

The marked difference of peramine levels in guttation fluid between tall fescue and perennial ryegrass could suggest that peramine fulfils a different ecological role in these two species. Peramine is not toxic to insects, but is a strong feeding deterrent (Rowan et al., 1986). A common pest of

Table 1
Grass species with their endophyte association and peramine concentration in fluids

Plant	Endophyte	Peramine (ng ml^{-1}) ± standard deviation ($n = 3$)		Ratio intact versus cut
		Cut leaf fluid	Guttation fluid	
Perennial ryegrass (*L. perenne*)	None	ND[a]	ND[a]	
Perennial ryegrass (*L. perenne*)	*Epichloë festucae* (Fl1)	567 ± 135	158 ± 117	0.28
Perennial ryegrass (*L. perenne*)	*Neotyphodium loili*	608 ± 295	554 ± 248	0.91
Tall fescue (*F. arundinacea*)	*Neotyphodium coenophialum*	524 ± 132	ND[a]	0
Elymus sp. BZ2155 high peramine[b]	*Epichloë* sp.[c]	4971 ± 1517	324 ± 232	0.04
Elymus sp. BZ2157 low peramine[b]	*Epichloë* sp.[c]	157 ± 70	7 ± 3	0.07

[a] Not detected. The limit of detection 0.8 ng ml^{-1}.

[b] These associations were selected for their extreme peramine levels. BZ2155 shows the highest peramine levels recorded, BZ2157 shows very low levels of peramine.

[c] Species is not fully identified and assumed to be a *Epichloë* sp.

perennial ryegrass in New Zealand is the Argentine stem weevil. It has been shown that adult argentine stem weevils prefer to sit on endophyte free ryegrass versus endophyte infected, without even eating the plant (Gerard, 2000), so that the exuded peramine on the outside of the plant may be detering adult weevils. However, it is likely that the concentration of peramine found in guttation fluid does not yield enough peramine to have a feeding deterrent effect on insects. The minimal concentration of peramine that shows any effect is at least 0.1 $\mu g\, g^{-1}$ plant material (Rowan et al., 1990). This would mean that wild type perennial ryegrass would need 0.2 ml of guttation to protect 1 g of plant material. Generally we were not able to collect more than 0.1 ml (often less) of guttation fluid from one tiller, which weighed excesses the 1 g level.

The tall fescue *N. coenophialum* association produces loline alkaloids in high concentration as well as ergot peptide alkaloids, and the guttation fluids contained detectable levels of both these alkaloid classes. We could clearly detect *N*-acetylloline and *N*-formylloline and their fragmentation is discussed elsewhere (in preparation). The concentration of lolines in guttation fluid ranged from 1 to 10 $\mu g\, ml^{-1}$. Because of the lack of sufficient literature on the concentration dependency of the anti-insect activity of lolines (Wilkinson et al., 2000), it is impossible to estimate if the levels of lolines in guttation fluid would cause any serious effects on insects.

The ergot peptide alkaloid ergovaline was identified in the tall fescue *N. coenophialum* association on the basis of its mass spectrometric fragmentation pattern (Lehner et al., 2005) and coelution with a standard and its concentration was around 0.01 and 0.1 $\mu g\, ml^{-1}$ (calculated as ergotamine). Ergovalinine and dehydroergovaline were tentatively identified in the cut leaf fluid on the basis of the m/z and rentention time, but we did not obtain fragmentation data to confirm their identity. The levels of ergovaline and lolines in guttation fluid were about 0.01 and 0.02 that of the levels in cut leaf fluid, respectively. Although perennial ryegrass infected with *Neotyphodium lolii* or *Epichloë festucae* also accumulate ergopeptide alkaloids, we could not detect any ergot alkaloids in the fluids from these associations. In contrast to tall fescue, perennial ryegrass does not seem to mobilize ergot alkaloids, but it is not clear to what extent this is controlled by an active process. Peramine and the ergot alkaloids therefore might have different ecological functions in these two plant species.

We are not aware of any previous report on the exudation of bioactive metabolites by the guttation process. Similarly, there is very little data on the mobilization of endophyte metabolites in the plant although several reports hypothesize that this takes place (Wilkinson et al., 2000; Lehtonen et al., 2005). As these mechanisms possibly play a role in the ecological function of the endophyte alkaloids *in planta* they deserve further attention.

The concept that plants have the capacity to exude ecologically functional compounds through guttation fluid is new to science. Many (if not all) plant species have hydathodes and in many climates a high humidity at night means guttation occurs on a frequent basis. Because the main constituent of guttation fluid is water, it is an ideal carrier for hydrophilic bioactives, which are too polar to diffuse through the cuticullar wax of the leaf surface (Müller and Riederer, 2005). For example guttation could explain the presence of hydrophyllic glucosinolates on the leaf surface of Cruciferae (Griffiths et al., 2001). The findings in this paper suggest the ecological significance of the exudation of bioactive metabolites via guttation fluid warrants further research.

3. Materials and methods

3.1. Plant material

Grass plants with or without endophyte were grown in a potting mix supplemented with Imidacloprid (Bayer, NZ) in individual pots in the greenhouse. The original seed lines were obtained from Margot Forde Forage Germplasm Centre, AgResearch Grasslands, Palmerston North, New Zealand. The majority of the associations used in this study were naturally infected, being grown from infected seeds (perennial ryegrass Nui 5 (*Lolium perenne*) with *Neotyphodium lolii* (WT, strain AR93), tall fescue (*Festuca arundinacea*) with *Neotyphodium coenophialum* and the 2 *Elymus* sp. with *Epichloë* sp.). The association of perennial ryegrass (*L. perenne*) G1057 with *Epichloë festucae* Fl1, was obtained by inoculating endophyte-free clonal plantlets. The endophyte-free plants used in this study are descendants of plants that were obtained by treating infected tillers with Benomyl fungicide. All the plants were regularly checked for the presence or absence of the expected endophyte.

To collect fluid, plants were placed overnight in a closed container. Before placement in the container some leaves were cut with scissors, approximately 5 cm from the leaf tip. In early morning the fluid accumulated at the leaf ends of one plant was collected with a pipette, transferred to a plastic container, and stored at −20 °C until analysis. For all fluid collections multiple plants were used. The range of plants available for collections varied. With most combinations several different infected plants of the same cultivar were used while with others clones of a single infected genotype were used.

4. LCMSMS

LCMSMS: All solvents used for LCMSMS were HPLC grade; solvents used for other procedures were of HPLC or analytical grade. For LCMSMS analysis the samples were thawed and transferred to an HPLC vial with 200 µl insert. Samples were kept at 5 °C in the autosampler, and 10 µl subsamples were injected. Analytes were eluted through a C18 Luna column (Phenomenex Torrence, CA, USA)

(150 × 2 mm, 5 μm) at a flow rate of 200 μl min^{-1} using a Thermo Finnigan Surveyor HPLC system with a solvent gradient (solvent A: H_2O 0.1% formic acid; B: MeCN 0.1% formic acid), starting with 3% B, 97% A for 5 minutes and then increasing to 23% B over 15 min followed by a column wash at 95% B.

Mass spectra were determined with a linear ion trap mass spectrometer (Thermo LTQ) using ESI in +ve mode. The spray voltage was 5.0 kV and the capillary temperature 275 °C. The flow rates of sheath gas, auxiliary gas, and sweep gas were set to 20, 5, and 10 (arbitrary units), respectively. Other parameters were optimized automatically by infusing peramine in H_2O:MeCN:HCOOH (95:5:0.1, v/v/v) at a flow rate of 200 μl min^{-1}. Peramine was detected in SRM mode, selecting 248.2 $m/z \pm 2$, 25% relative collision energy, and quantifying the 206.2 m/z fragment ion. For comparison the UV spectra were obtained using a Thermo surveyor PDA detector (200–600 nm). We used a six point calibration curve ranging from 10 μg ml^{-1} to 1 ng ml^{-1} of peramine dissolved in water. During routine analysis of peramine we used a three point calibration curve flanked by a blank before and after every five samples. A pooled sample of guttation fluid was used as a control sample to monitor inter-day variation.

Peramine: Rt:12.4 min; ESIMS (positive ion mode; m/z, rel int (%)): m/z 248.2 $[M + H]^+$ ms2 248.20 @ 35% CE: 231.2 (16.4), 206.2 (100), 175.2 (6.4), 174.2 (3.5) 149.2 (1.6), ESIMS ms3 248.20 @ 35% CE, 231.20@ 35% CE: 214.2 (3.9), 200.2(50.8), 175.2 (19.9) 174.2 (100) ESIMS ms3 248.20 @ 35% CE, 206.10 @ 35% CE: 175.2 (100), 149.10732 (27.4).

Imidacloprid: Rt: 24.3 min; ESIMS (positive ion mode; m/z, rel int (%)): m/z 256.1 $[M + H]^+$ ms2 256.1 @ 35% CE: 226.0 (5.9), 212.0 (61.1), 210.0 (100), 209.1 (76.6) 175.1 (46.4) 174.1 (10.0), 128.0 (2.1), 84.1 (3.1) ESIMS ms3 256.10 @ 35% CE, 210.10 @ 35% CE: 193.1 (1.9), 175.0 (100), 174.0 (26.1), 166.9 (1.9), 84.0 (1.9) (Laurent and Rathahao, 2003).

4-Hydroxy-imidacloprid: Rt: 22.1 min; ESIMS (positive ion mode; m/z, rel int (%)): m/z 272.1 $[M + H]^+$; ms2 272.10 @ 35% CE: 242.0 (2.48), 228.0 (100), 226.1 (40.7), 225.0 (36.0), 210.0 (4.35), 209.0 (2.2), 191.1 (25.9), 190.0 (28.8); ESIMS ms3 272.10 @ 35% CE, 228.00 @ 35% CE: 210.0 (100), 168.9 (13.3), 167.1 (10.2), 144.0 (63.4), 128.0 (23.1), 126.0 (42.4) (Laurent and Rathahao, 2003).

Ergovaline : Rt: 24.8 min; ESIMS (positive ion mode; m/z, rel int (%)): m/z 534.2 $[M + H]^+$ ms2 534.2 @ 35% CE: 516.1 (100), 491.0 (1.4), 419.2 (1.1), 320.1 (1.5), 277.1 (4.6), 268.2 (2.6), 223.1 (0.8), 207.9 (0.7), 191.2 (0.2); ESIMS ms3 534.20 @ 35% CE, 516.10 @ 35% CE: 488.2 (23.1), 320.1 (49.1), 277.0 (50.5), 268.1 (100), 249.1 (52.4), 223.1 (31.9), 221.1, (6.9), 208.1 (4.0), 207.1 (3.1), 192.2 (2.8), 191.1 (1.2) (Lehner et al., 2005).

N-acetylloline Rt: 2.1 min; ESIMS (positive ion mode; m/z, rel int (%)): m/z 197.3 $[M + H]^+$ ms2 197.3 @ 35% CE: 179.1 (4.1), 155.1 (100), 154.0 (76.1), 141.1 (3.1), 138.1 (2.6), 135.2 (1.6), 124.1 (6.5), 112.1 (5.1), 99.0 (3.3); ESIMS ms3 197.30 @ 35% CE, 155.10 @ 35% CE: 138.1 (1.2), 126.0 (4.1), 124.0 (51.3), 112.0 (100), 95.0 (2.3), 82.0 (2.2).

N-formylloline Rt: 1.8 min; ESIMS (positive ion mode; m/z, rel int (%)): m/z 183.3 $[M + H]^+$ ms2 183.25 @ 35% CE: 165.0 (2.34), 155.1 (100), 124.1 (1.8), 112.1 (2.4); ESIMS ms3 183.25 @ 35% CE, 155.10 @ 35% CE: 138.1 (1.3), 126.0 (4.4), 124.0 (50.7), 112.0 (100), 95.1 (2.5), 94.1 (0.5), 84.1 (1.6), 82.1 (2.3).

5. ESI FTICRMS

Accurate mass on peramine and its fragments from CID in an ion-trap was obtained on a Thermo Finnigan LTQ FT, which is a combination of a linear ion trap mass spectrometer and a Fourier transform ion cyclotron resonance mass spectrometer.

Peramine: ESIFTICRMS (positive ion mode): m/z 248.15063 $[M + H]^+$ (calc. for $C_{12}H_{18}O_1N_5$, 248.15059); ESIFTICRMS ms2 248.20 @ 35% CE: 231.12405 $[-NH3]^+$ (calc. for $C_{12}H_{15}O_1N_4$, 231.12404), 206.12877 $[-CH_2N_2]^+$ (calc. for $C_{11}H_{16}O_1N_3$, 206.124879), 175.08657 $[-C_2H_7N_3]^+$ (calc. for $C_{10}H_{11}O_1N_2$, 175.08659), 149.10732 $[-C_3H_5O_1N_3]$ (calc. for $C_9H_{13}N_2$, 149.10733); ESIFTICRMS ms3 248.20 @ 35% CE, 231.20 @ 35% CE: 200.08184 $[-C_1H_5N_1]^+$ (calc. for $C_{11}H_{10}O_1N_3$, 200.08184), 175.08657 $[-C_2H_4N_2]$ (calc. for $C_{10}H_{11}O_1N_2$, 175.08659), 174.10257 $[-C_2H_3O_1N_1]^+$ (calc. for $C_{10}H_{12}N_3$, 174.10257). ESIFTICRMS ms3 248.20 @ 35% CE, 206.10 @ 35% CE: 175.08657 $[-C_1H_5N_1]^+$ (calc. for $C_{10}H_{11}O_1N_2$, 175.08659), 149.10732 $[-C_2H_3O_1N_1]^+$ (calc. for $C_9H_{13}N_2$, 149.10733).

Acknowledgements

We acknowledge the support of the Foundation for Research Science and Technology, Contract C10X0203 *New opportunities from Forage Plant Genomics* and the AgResearch Board for Reinvestment funding for metabolomics. We thank David Greenwood (HortResearch and Auckland University) for his help with the ESIFTICRMS and Marit van Gils for her help with the collection and preparation of the plant fluids.

References

Ball, O.J.P., Barker, G.M., Prestidge, R.A., Lauren, D.R., 1997. Distribution and accumulation of the alkaloid peramine in Neotyphodium lolii-infected perennial ryegrass. Journal of Chemical Ecology 23, 1419–1434.

Bollard, E.G., 1960. Transport in the Xylem. Annual Review of Plant Physiology 11, 141–166.

Breen, J.P., 1994. Acremonium endophyte interactions with enhanced plant resistance to insects. Annual Review of Entomology 39, 401–423.

Bürkle, L., Cedzich, A., Döpke, C., Stransky, H., Okumoto, S., Gillissen, B., Kühn, C., Frommer, W.B., 2003. Transport of cytokinins mediated

by purine transporters of the PUP family expressed in phloem, hydathodes, and pollen of Arabidopsis. Plant Journal 34, 13–26.

Bush, L.P., Wilkinson, H.H., Schardl, C.L., 1997. Bioprotective alkaloids of grass-fungal endophyte symbioses. Plant Physiol 114, 1–7.

Fabricant, D.S., Nikolic, D., Chen, S.N., Van Breemen, R.B., Fong, H.H.S., Farnsworth, N.R., Pauli, G.F., Jaki, B.U., Lankin, D.C., Krunic, A., 2005. Cimipronidine, a cyclic guanidine alkaloid from Cimicifuga racemosa. Journal of Natural Products 68, 1266.

Fletcher, L.R., 2005. Managing ryegrass-endophyte toxicoses. In: Roberts, C.A., West, C.P., Spiers, D.E. (Eds.), *Neotyphodium* in Cool-Season Grasses. Blackwell Publishing, Ames, USA, pp. 229–241.

Gerard, P.J., 2000. Ryegrass endophyte infection affects Argentine stem weevil adult behaviour and susceptibility to parasitism. New Zealand Plant Protection 53, 406–409.

Griffiths, D.W., Deighton, N., Birch, A.N.E., Patrian, B., Baur, R., Stadler, E., 2001. Identification of glucosinolates on the leaf surface of plants from the Cruciferae and other closely related species. Phytochemistry 57, 693.

Keogh, R.G., Tapper, B.A., Fletcher, R.H., 1996. Distributions of the fungal endophyte Acremonium lolii, and of the alkaloids lolitrem B and peramine, within perennial ryegrass. New Zealand Journal of Agricultural Research 39, 121–127.

Laurent, F.M., Rathahao, E., 2003. Distribution of [C-14]imidacloprid in sunflowers (*Helianthus annuus* L.) following seed treatment. Journal of Agricultural and Food Chemistry 51, 8005–8010.

Lehner, A.F., Craig, M., Fannin, N., Bush, L., Tobin, T., 2005. Electrospray[+] tandem quadrupole mass spectrometry in the elucidation of ergot alkaloids chromatographed by HPLC: Screening of grass or forage samples for novel toxic compounds. Journal of Mass Spectrometry 40, 1484–1502.

Lehtonen, P., Helander, M., Wink, M., Sporer, F., Saikkonen, K., 2005. Transfer of endophyte-origin defensive alkaloids from a grass to a hemiparasitic plant. Ecology Letters 8, 1256.

Müller, C., Riederer, M., 2005. Plant surface properties in chemical ecology. Journal of Chemical Ecology 31, 2621.

Pilot, G., Stransky, H., Ludewig, U., Frommer, W.B., Bushey, D.F., Wingate, V.P.M., Pratelli, R., 2004. Overexpression of GLUTAMINE DUMPER1 leads to hypersecretion of glutamine from hydathodes of arabidopsis leaves. Plant Cell 16, 1827–1840.

Popay, A.J., Bonos, S.A., 2005. Biotic responses in endophytic grasses. In: Roberts, C.A., West, C.P., Spiers, D.E. (Eds.), *Neotyphodium* in Cool-Season Grasses. Blackwell Publishing, Ames, USA, pp. 164–185.

Rowan, D.D., Dymock, J.J., Brimble, M.A., 1990. Effect of fungal metabolite peramine and analogs on feeding and development of argentine stem weevil (*Listronotus bonariensis*). Journal of Chemical Ecology 16, 1683–1695.

Rowan, D.D., Gaynor, D.L., 1986. Isolation of feeding deterrent s against stem weevil from ryegrass infected with the endophyte *Acremonium loliae*. Journal of Chemical Ecology 12, 647–658.

Rowan, D.D., Hunt, M.B., Gaynor, D.L., 1986. Peramine, A novel insect feeding deterrent from ryegrass infected with the endophyte Acremonium-Ioliae. Journal of The Chemical Society – Chemical Communications, 935–936.

Siegel, M.R., Latch, G.C.M., Johnson, M.C., 1985. Acremonium fungal endophytes of tall fescue and perennial ryegrass – significance and control. Plant Disease 69, 179–183.

Spiering, M.J., Davies, E., Tapper, B.A., Schmid, J., Lane, G.A., 2002. Simplified extraction of ergovaline and peramine for analysis of tissue distribution in endophyte-infected grass tillers. Journal of Agricultural and Food Chemistry 50, 5856–5862.

Spiering, M.J., Lane, G.A., Christensen, M.J., Schmid, J., 2005. Distribution of the fungal endophyte *Neotyphodium lolii* is not a major determinant of the distribution of fungal alkaloids in *Lolium perenne* plants. Phytochemistry 66, 195–202.

Taiz, L., Zeiger, E., 1991. Plant Physiology. The Benjamin/Cummings Publishing Company Inc., Redwood City, Ca.

Wilkinson, H.H., Siegel, M.R., Blankenship, J.D., Mallory, A.C., Bush, L.P., Schardl, C.L., 2000. Contribution of fungal loline alkaloids to protection from aphids in a grass-endophyte mutualism. Molecular Plant–Microbe Interact 13, 1027–1033.