

Wade J. Mace¹
Chris G. L. Pennell²
M. Phil Rolston²
Tony J. Hilditch²
Stuart D. Card¹



¹AgResearch Ltd.,
Grasslands Research Centre,
Palmerston North, New Zealand

²AgResearch Ltd.,
Lincoln Research Centre,
Christchurch, New Zealand

agresearch

wade.mace@agresearch.co.nz

Investigating potential transfer of endophyte-grass toxins to horticultural crops

Methods

Soil

Soil samples were taken from under two established areas growing either

tall fescue (*Lolium arundinacea*) cv 'Jackal' with *Epichloë coenophiala* strain AR601 (TF E+)

or

perennial ryegrass (*Lolium perenne*) cv. 'Colosseum' with *Epichloë festucae* var. *lolii* strain AR95 (PRG E+).

The three control soil samples:

tall fescue with no endophyte (TF E-)

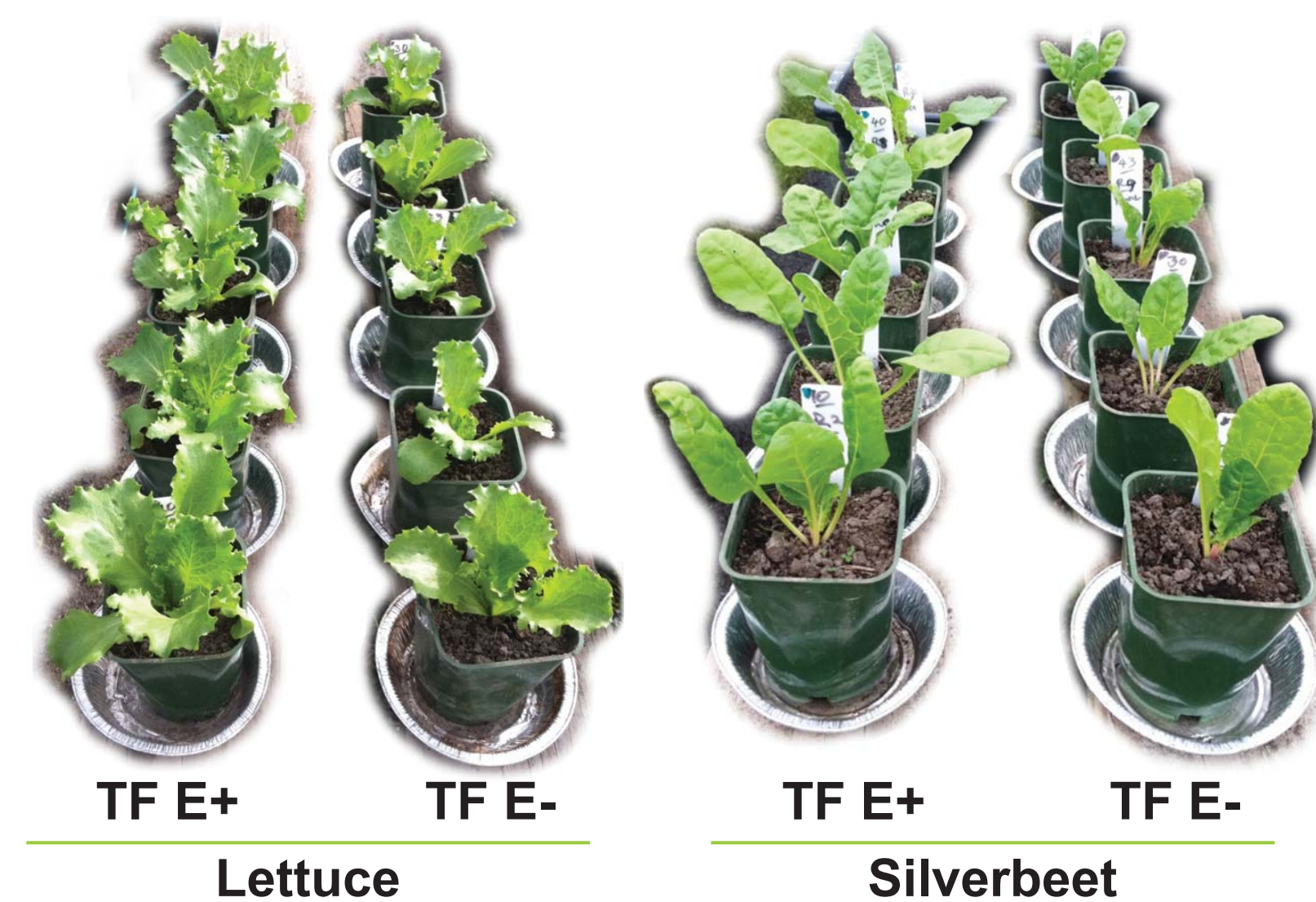
perennial ryegrass with no endophyte (PRG E-)

bare soil where no plants were grown for three years.

Alkaloid analyses

Plant material was analysed by HPLC and GC-FID for lolitrem B, ergovaline, peramine and total lolines using published protocols^{3,4}.

Initial analyses were conducted on bulks of replicates from individual treatments, with some experiments selected for analysis of individual replicates.



Experimental setup for potatoes



Field of haricot beans at Lincoln prior to harvest



Plants

Barley, carrot, lettuce, and silverbeet were raised from seed and grown in a glasshouse in pots containing soils as detailed.

Seed potatoes were planted into large gauze-bottomed pots containing soil of the corresponding treatments, and placed outside on a sand pad.

All plants received water and nutrients as required until the plants reached maturity, at which time the plants were harvested and the plant tissue of interest analysed for endophyte alkaloids.

Haricot beans were harvested from a crop grown at Lincoln on an area that had grown Jackal AR601 for the preceding three years.

| Common name | Species | Plant tissue analysed | Plants raised in | Replication |
|--------------|--|-----------------------|------------------|-------------|
| Lettuce | <i>Lactuca sativa</i> | Leaf | Pots | n = 10 |
| Potato | <i>Solanum tuberosum</i> | Tuber | Pots | n = 10 |
| Carrot | <i>Daucus carota</i> subsp. <i>sativus</i> | Taproot | Pots | n = 10 |
| Silverbeet | <i>Beta vulgaris</i> subsp. <i>cicla</i> | Leaf | Pots | n = 10 |
| Barley | <i>Hordeum vulgare</i> | Grain | Pots | n = 10 |
| Haricot bean | <i>Phaseolus vulgaris</i> | Bean | Field | n = 6 |

Introduction

Associations between Pooideae grasses and *Epichloë* fungal endophytes are utilised in agriculture and recreation areas for the benefits the associations provide in pasture persistence and performance.

This includes the wild-life deterrent grass-endophyte product Avanex® Unique Endophyte Technology¹ which is used in amenity areas, sports grounds and airports for the deterrent effects the endophyte alkaloids have on insect, bird and small mammalian pests.

Mammalian toxins produced by grass-endophyte associations have been detected in soil supporting tall fescue pastures² with suggestions that these compounds could remain within the soil matrix for an extended time.

As changes in land use can occur, there is the potential for arable and horticultural crops to be grown in soil previously supporting grass-endophyte associations, thereby potentially absorbing endophyte alkaloids. Therefore, it is necessary to undertake scientific evaluations to provide assurances that soil is 'safe', especially if the subsequent crop is for animal or human consumption.

Aim

To investigate whether food crops could absorb endophyte alkaloids from soil that had been exposed to Avanex® Unique Endophyte Technology.

Acknowledgements

Technical support was provided by Xinqi Liu (AgResearch Ltd).

Funding was provided by the Foundation for Arable Research (FAR), Grasslanz Technology Limited and PGG Wrightson Seeds Limited.

Avanex® Unique Endophyte Technology is a registered trademark of PGG Wrightson Seeds Limited.



References

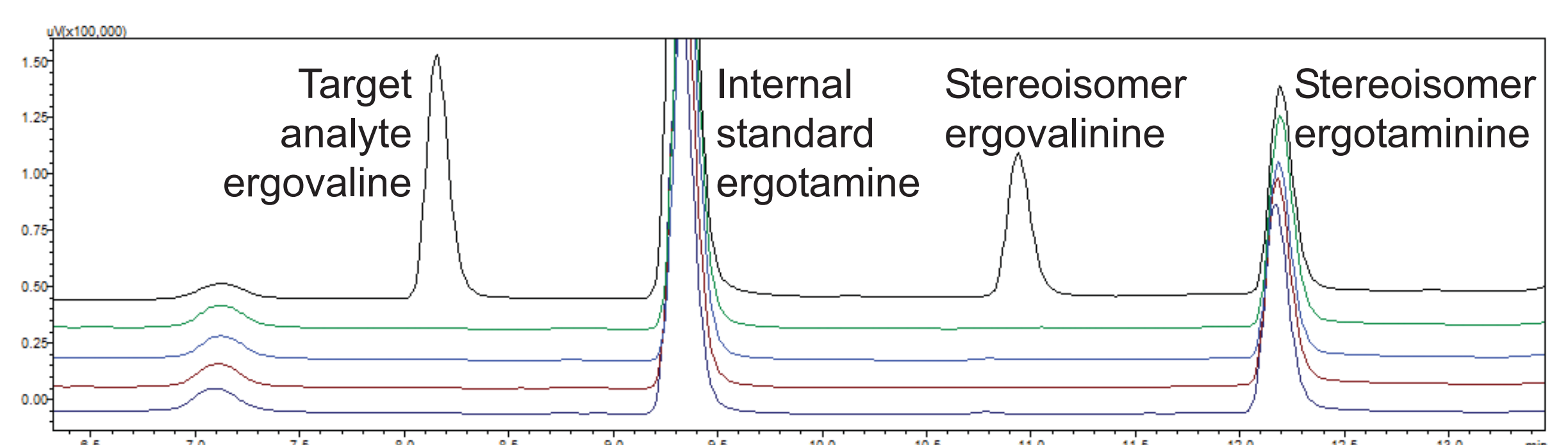
- Pennell, C., Rolston, M., de Bonth, A., Simpson, W., & Hume, D. (2010). Development of a bird-deterrent fungal endophyte in turf tall fescue. *New Zealand Journal of Agricultural Research*, 53(2), 145 - 150.
- Franzluebbers, A. J., & Hill, N. S. (2005). Soil carbon, nitrogen, and ergot alkaloids with short- and long-term exposure to endophyte-infected and endophyte-free tall fescue. *Soil Science Society of America Journal*, 69(2), 404-412. doi: 10.2136/sssaj2005.0404
- Baldauf, M. W., Mace, W. J., & Richmond, D. S. (2011). Endophyte-mediated resistance to Black Cutworm as a function of plant cultivar and endophyte strain in tall fescue. *Environmental Entomology*, 40(3), 639-647. doi: 10.1603/en09227
- Reed, K. F. M., Nie, Z. N., Walker, L. V., Mace, W. J., & Clark, S. G. (2011). Weather and pasture characteristics associated with outbreaks of perennial ryegrass toxicosis in southern Australia. *Animal Production Science*, 51(8), 738-752. doi: 10.1071/an11016

Results

There was no evidence for the presence of lolitrem B, ergovaline, peramine or the lolines in crops grown in soil that had previously supported endophyte-infected pasture.

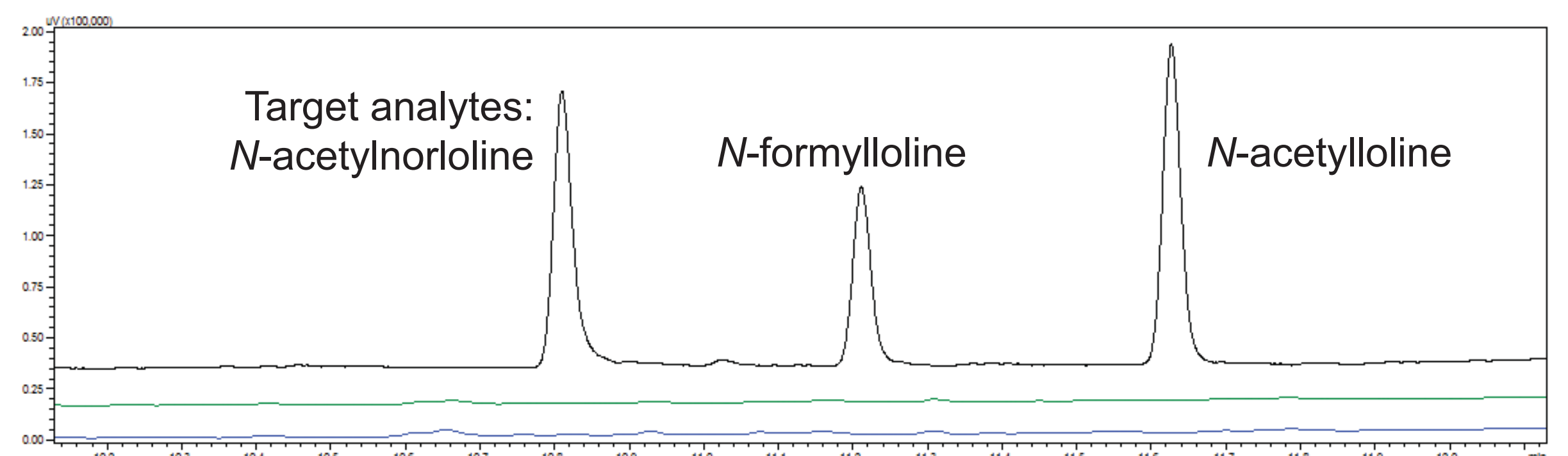
Ergovaline chromatograms

Ergovaline HPLC chromatogram of silverbeet leaves grown in Jackal AR601 soil (green), tall fescue E- soil (blue), Colosseum AR95 soil (brown), Colosseum E- soil (dark blue). Reference standard (black).



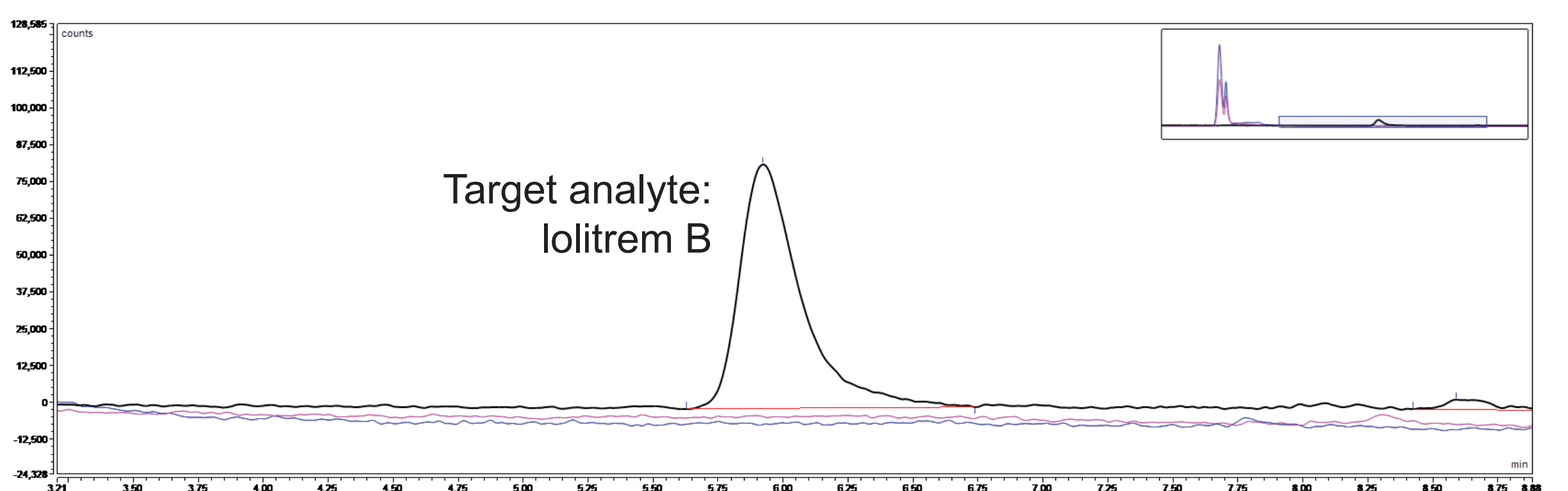
Lolines chromatograms

Lolines GC-FID chromatogram of carrot grown in Jackal AR601 soil (green), endophyte free soil (blue). Reference standard (black).



Lolitrem B chromatograms

Lolitrem B HPLC chromatogram of barley grown in Colosseum soil (blue), nil soil (pink). Reference standard (black).



Discussion

There is no evidence to support the contamination of crops with ergovaline, lolitrem B, lolines, or peramine from soils that have grown endophyte-infected pasture. This indicates that the rotation between pasture seed production and cropping will not lead to endophyte alkaloids getting into the animal or human food chains.

It should also be noted that no attempt was made to determine the levels of endophyte alkaloids present in the soils. This work was undertaken to establish whether crops grown on soil previously used to grow endophyte-infected pasture grasses had detectable levels of endophyte alkaloids and toxins.

There are three possible explanations for the absence of detection of endophyte toxins in the crops; absence of endophyte alkaloids in the soil matrices, inability of the crop plants to effectively absorb detectable quantities of the endophyte alkaloids, modification/degradation of the alkaloids once absorbed by the crop plants.

Future work should establish the level and range of endophyte alkaloids in soil through a survey across a range of locations under a range of land uses.