Validation of ergot alkaloid assay methods

Summary
The discovery of sorghum ergot in Australia in 1996 was a very serious issue for the sorghum industry. This fungus (*Claviceps africana*) infects the plant at flowering, and after a growth cycle featuring the release of sticky honeydew, it produces small hard fungal bodies called sclerotia (ergots) in place of seed. These ergots contain alkaloids which are poisonous to livestock, causing reduced milk production, reduced growth and impaired heat tolerance. Effective methods were needed to detect and measure the alkaloids present in grain in order to assess suitability for various markets. Another type of ergot, rye ergot (*Claviceps purpurea*) is an occasional contaminant of wheat and barley in parts of New South Wales, and also has caused serious losses in livestock production. Rye ergot produces a different range of alkaloids than sorghum ergot.

Two assay methods for sorghum ergot alkaloids (mainly dihydroergosine), based on High-Performance-Liquid-Chromatography (HPLC) and on Enzyme-Linked-Immunosorbant-Assay (ELISA) were developed to a preliminary stage in a previous project supported by GRDC (DAQ428). The current project was established in order to further develop the assays to a stage where they could be used by other laboratories, and to fully document and validate them. Subsequently, the project was expanded slightly to assess whether the methods developed for sorghum ergot could also be used to assure the quality of wheat and barley in respect to rye ergot, and the completion date was postponed for 12 months until December 2003, so as to include testing of the 2003 crop.

Specifically, the aims of this project were:

- To package and adapt sorghum ergot alkaloid assay methods to suit the actual conditions required by end-users including commercial laboratories, feed manufacturers, bulk grain handlers and growers;
- To supply a selection of these end-users with reference materials and standards over the 2001/02 sorghum seasons, to verify results obtained by those users with different methods; and
• To provide a reference service for addressing any inconsistencies between test results; and to combine available alkaloid assay methods with appropriate risk minimisation processes to assist in ensuring that sorghum meets regulatory and other end-user requirements.

Report Disclaimer
This document has been prepared in good faith on the basis of information available at the date of publication without any independent verification. Grains Research & Development Corporation (GRDC) does not guarantee or warrant the accuracy, reliability, completeness or currency of the information in this publication nor its usefulness in achieving any purpose. Readers are responsible for assessing the relevance and accuracy of the content of this publication. GRDC will not be liable for any loss, damage, cost or expense incurred or arising by reason of any person using or relying on information in this publication. Products may be identified by proprietary or trade names to help readers identify particular types of products but this is not, and is not intended to be, an endorsement or recommendation of any product or manufacturer referred to. Other products may perform as well or better than those specifically referred to. Check www.apvma.gov.au and select product registrations listed in PUBCRIS for current information relating to product registration.

Copyright
Grains Research and Development Corporation. This publication is copyright. Apart from any use as permitted under the Copyright Act 1968, no part may be reproduced in any form without written permission from the GRDC.

Old or Archival Reports (Projects that concluded in 2007 or earlier)
The information contained in these older reports is now several years old, and may have been wholly or partially superseded or built upon in subsequent work funded by GRDC or others. Readers should be aware that more recent research may be more useful for their needs. Findings related to agricultural chemical use are also potentially out of date and are not to be taken as a recommendation for their use.

Conclusions
The project successfully developed and validated effective assay methods for ergot alkaloids in sorghum that can also be applied to rye ergot alkaloids in wheat and barley. These methods were fully documented and published and their availability widely promulgated in various news media. The assays were used for testing grain during the 2001-2003 seasons.

The project did not succeed in getting the method established in other laboratories. The main commercial laboratories serving the industry expressed interest at the start of this project, but have since declined to take on the assay, because demand has been deemed too small. For the same reason, a commercial manufacturer of ELISA kits has not been interested in developing and marketing the ergot ELISA. This risk was forecast in the project proposal.

The low incidence of contamination is considered to be largely due to the effectiveness of the extension campaign mounted by Department of Primary Industries (DPI) about the high risk of ergot contamination in late-planted crops. Experience over the past five years suggests that, unless climatic conditions change markedly, the concentration of sorghum ergot is likely to remain very low in bulk grain, but there will be always be a few crops seriously affected that require assay. A similar scenario applies to rye ergot in wheat.

One factor affecting demand for testing is that current regulatory standards specify 0.3% ergot, rather than the alkaloids themselves, and so there is more demand for ergot testing than alkaloid testing. This might change if the regulations are amended to specify limits for alkaloid, as is being considered.

Recommendations
That the DPI maintains the availability of ergot alkaloid assays to industry for the foreseeable future, and transfers the
methods to any other laboratory that might express interest in the future.

**Other research**
Technology for packaging ELISA tests into robust kits for use outside the laboratory are continually under development (e.g. card tests). While the present market for these tests appears too small for commercial development, opportunities could develop in the future.

**Intellectual property summary**
The DPI holds monoclonal antibodies on which the ELISA tests for sorghum and rye ergot alkaloids are based. These are essential for any future commercialisation of these tests, and consequently have intellectual property value.

**Additional information**