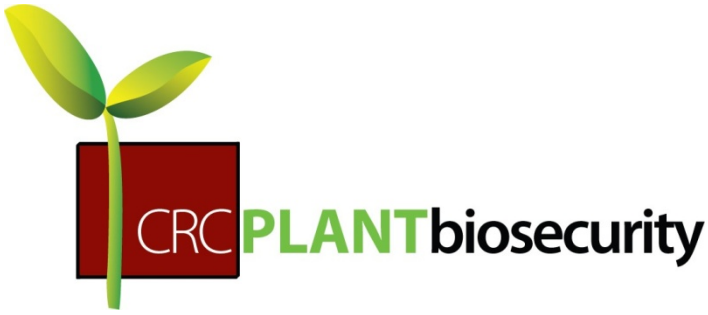

Cooperative Research Centre
for National Plant Biosecurity

Final Report

CRC20137

Khapra beetle Diagnostics

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1. Executive Summary

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is recognised as one of the world's most destructive pests of grain products and is subject to strict quarantine measures in many countries. Plant Health Australia has identified khapra beetle as one of the top five biosecurity threats to the Australian Grains Industry (AGI).

Australia is currently free of khapra beetle and in order to ensure this status is maintained it is necessary to detect and recognise the species quickly should it find its way to our shores. Unfortunately khapra beetle is one among a 'fog' of similar related species. This, and the inherent difficulties of identifying Dermestids (requiring considerable experience and usually dissection and examination of genitalia) makes identification slow, difficult, and requiring special care to avoid misidentifications.

The 'Khapra beetle Diagnostics' project (CRC20137) has conducted research and other activities to better understand and improve on the diagnostics of khapra beetle, and relatives, in Australia:

1. A national trapping programme
2. Development of diagnostic protocols, keys and images to support identification of Dermestids
3. Development and validation of molecular diagnostic tools (ongoing work from CRC60046) which promises reliable and fast batch screening for khapra beetle
4. Established a *Trogoderma* and Related Dermestids reference laboratory to conduct and support the aforementioned activities

Hopefully the contributions of this project will mean that the AGI is well placed to ensure that when khapra beetle makes its way to Australia it can be sought out and eradicated quickly, and not be allowed to "sneak" across Australia as an unrecognised wolf in sheep's clothing.

2. Aims and objectives

2.1. Rationale

The essential problem this project deals with is sometimes referred to as 'The khapra beetle problem'. The khapra beetle problem is, in essence, that khapra beetle is a serious pest of stored grain and in countries free of khapra beetle, such as Australia, the detection and recognition of khapra beetle is a critical but difficult task. Surveillance is needed to detect suspect khapra beetle and then there are diagnostic difficulties which make recognising that khapra beetle has been collected difficult. There are many species similar to khapra beetle so making its recognition a task akin to recognising a wolf in sheep's clothing.

2.2. Aims (Milestones and Deliverables)

A general statement of the aim of the project is to address the 'khapra beetle problem', in particular by:

1. gaining a better understanding of taxonomy of the taxa
2. developing new diagnostic tools (images, keys, molecular tools)
3. capacity building (training, extension, collections)

Below are the details of the assorted specific milestones and deliverables of the CRC.

Milestones

1. Project signed and loaded in iMap
2. Australian Dermestid and *Trogoderma* collections reviewed; pilot *Trogoderma* trapping programme completed
3. American and European Dermestid and *Trogoderma* collections reviewed; full national *Trogoderma* trapping program completed
4. Comparative taxonomy of overseas and Australian *Trogoderma* consolidated
5. PaDIL faceted keys and PaDIL images available; second full national *Trogoderma* trapping programme completed
6. National Dermestid Reference laboratory operational
7. Final report submitted to CRCNPB including completion of Post Completion Survey

Deliverables

1. DNA-based and/or proteomic test kits for the rapid identification of *Trogoderma* species
2. A national trapping program for the surveillance of khapra beetle
3. A National Reference Laboratory for the identification of Dermestid beetles

2.3. Reporting approach

Rather than reporting against the particular temporal milestones outlined above we have opted to report the activities and results (subject focused approach) of the project in four parts, *viz.*

1. Establishment of a National Reference Laboratory for *Trogoderma*
2. National trapping programme
3. Taxonomy
4. Molecular diagnostics

In each section an indication is given as to which milestones the reported issues relate.

The work covered in each of these sections addresses the assorted milestones and deliverables, with additional technical depth being covered in some technical addenda, and other specific milestone-related issues having been reported through iMap.

2.4. Project beneficiaries

The principal beneficiaries of the project's research are:

1. Grains industry generally
2. Biosecurity agencies generally
3. DAFWA

3. Key findings

3.1. Establishment of a National Reference Laboratory for Trogoderma

Relates to MS6

An important goal of the project was to establish 'a National Reference Laboratory for *Trogoderma* and related Dermestids, to provide diagnostic capacity services, training and a platform for future surveillance and incursion management projects'.

The range of activities conducted by the project, summarised in the following, together constitute work of the National Reference Laboratory with the resources and capabilities to provide diagnostic services, training, surveillance for *Trogoderma* and related Dermestids.

Herein we refer to this laboratory as TARDIS (*Trogoderma* And Related Dermestids Identification Service).

3.2. National trapping programme

Relates to MS2, 3, 5, 6

A National Trapping Programme (NTP) for *Trogoderma* and Related Dermestids was carried out in collaboration with CBH, GrainCorp, and Viterra Inc (formerly ABB Grain Ltd) at 91 sites across the major grain production and handling regions of Australia between October 2009 and October 2011.

Collaborators maintained four traps (two Unitraps and two Khapra beetle traps) baited with pheromone and/or food lures at each site. Monthly the trap contents, or entire trap, were returned to DAFWA for processing.

A total of 1196 traps were returned with 9435 *putative* Dermestid specimens. Of these Dermestids 2773 specimens has been examined closely and specimens identified to genus or species with three species (*Anthrenus verbasci*, *Attagenus fasciatus* and *Trogoderma variabile*) and five genera (*Anthrenocerus*, *Anthrenus*, *Attagenus*, *Orphinus* and *Trogoderma*) being formally identified from the material.

The remainder of the specimens have undergone sorting to morphospecies with more than 95% of material sorted to Recognisable Taxonomic Unit (RTU) and these will continue to be identified as well as being used for molecular diagnostic experiments, specimens for imaging, for further study of Dermestid biodiversity and other uses.

No *T. granarium* have been found among the NTP specimens and so provides further support for Australia's freedom from this pest.

3.3. Taxonomy

Relates to MS2, 3, 4, 5, 6

The TARDIS taxonomist Andras Szito visited ten major collections in eight countries in North America and Europe during the project. During these visits specimens and literature were examined and assorted matters discussed with fellow taxonomists.

Additionally Andras has continued work on the development of the "ISPM 27 Diagnostic Protocol Draft for *Trogoderma granarium* Everts" as part of the development of diagnostic tools.

A two week course in the diagnostics of Dermestids was held by him for five participants in Canberra during May 2009 at the Australian National Insect Collection (ANIC).

More complete details of "Taxonomy" work are provided in an addendum. This addendum includes:

1. details of the institutions and specimens examined on the study tours
2. diagnostic details (including keys, images, specimen handling protocols) for Khapra beetle and related Dermestids
3. details of the training course and other training materials developed

3.4. Molecular diagnostics

Relates to MS6

TARDIS examined several aspects of the molecular diagnostics of khapra beetle, especially following from work in our sister project 'Khapra beetle Diagnostics' project (CRC20137).

In particular we examined the application of non-destructive DNA extraction

techniques, and (re)designed and partially validated species specific primer-probes for khapra beetle. In initial validation work the primer-probe has performed well.

Additionally a Molecular Diagnostic Laboratory (MDL) was established within DAFWA Entomology.

More complete details of “Molecular diagnostic” work are provided in an addendum. The addenda include:

1. a brief account of the establishment and capacities of the MDL
2. comparison of the effectiveness of several DNA extraction methods
3. full details of the design and test of the *diagnostic specificity* of the developed khapra beetle specific primer-probe

4. Implications for stakeholders

4.1. Establishment of a National Reference Laboratory for Trogoderma

TARDIS has the potential to provide critical diagnostic support for *all* stakeholders with an interest in protecting the AGI from khapra beetle and related exotic pests so stakeholders should ensure that the activities of TARDIS continue to develop and provide better tools and other resources for this task.

DAFWA has the opportunity to develop a centre of expertise around the, necessarily rare, expertise in the Dermestid family possessed by Andras Szito and supported by ongoing work to develop new and different tools (imaging, molecular diagnostics etc). Such a centre would not only provide critical support of the biosecurity of the grains industry but also give considerable kudos to DAFWA.

4.2. National trapping programme

The NTP has allowed a wide range of stakeholders to participate in a trapping programme for Dermestids.

The NTP should help encourage vigilance and generally awareness among the AGI relating to Dermestid pests, as well as the practicalities of a NTP.

The NTP has provided TARDIS with a wide range of native *Trogoderma* and other Dermestid specimens which will form the basis of further research to help address the ‘khapra beetle problem’.

4.3. Taxonomic work

Despite the efforts of TARDIS there are still many issues to be resolved around the taxonomy of khapra beetle and related Dermestids and so there is a need for ongoing basic work to study this taxon.

Additionally there are is a need to further develop diagnostic tools and provide training so that there is a greater general capacity for dealing with Dermestid diagnostic issues.

All stakeholders should recognise how critically important it is to be able to reliably identify khapra beetle and so support ongoing work in this area.

4.4. Molecular diagnostics

The khapra beetle specific primer-probe developed and partially validated performed well (to the extent tested), well enough that further work should be done to further the in-house validation of this primer-probe.

If the khapra beetle specific primer-probe performs well in further validations there is the opportunity to apply to test to mass screening of whole insect specimens (as collected in a trapping programme), and perhaps even, of detritus such as might be collected from the air around a grain handling facility.

If the khapra beetle specific primer-probe performed well in validations and could adopted for use with some level of mass screening this would represent a considerable 'breakthrough' in being able to identify khapra beetle quickly and with a high reliability with low technical skills. Of course, a critical part of any mass screening approach would include cross-validation, in this case morphological examination of some specimens by a highly skilled taxonomist, which is still the accepted 'gold standard'.

All stakeholders should be keen to pursue the considerable opportunities to develop and test a khapra beetle specific primer-probe further.

The importance of the MDL in Entomology should be recognised and it be supported for future research.

5. Recommendations

General recommendations related to each of the aspects of the project follow. Additionally those parts with technical addenda include more detailed specific recommendations.

5.1. TARDIS

1. TARDIS should be resourced and supported further to develop new tools and provide support for diagnostics of Dermestids
2. The diagnostic support TARDIS can provide should be made known to more widely and better leveraged as a collaborator with other projects or activities potentially collecting Dermestids. There are clear synergies in such collaborations with TARDIS experiencing a wider range of specimens and the collaborators receiving information regarding the taxa they are collecting

5.2. NTP

1. The material collected by the NTP should be identified further
2. The material should be used for ongoing research and other work, such as imaging, development of molecular tools, etc

5.3. Taxonomy

1. It is critical there be ongoing alpha taxonomic work done with *Trogoderma* and related Dermestids
2. More diagnostic tools and training should be provided to support capacity

building for the diagnostics of the Dermestids

5.4. Molecular diagnostics

1. Further work should be done to validate and further develop the application of the khapra beetle species-specific primer-probe

6. Abbreviations / glossary

6.1. Abbreviations

ABBREVIATION	FULL TITLE
AGI	Australian Grains Industry
ANIC	Australian National Insect Collection
CBH	Cooperative Bulk Handling
CRC	Cooperative Research Centre
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
DAFWA	Department of Agriculture and Food WA
DNA	Deoxyribonucleic Acid
GRDC	Grains Research and Development Corporation
ISPM	International Standards for Phytosanitary Measures
MDL	Molecular Diagnostics Laboratory
NTP	National Trapping Programme
PaDIL	Pest and Diseases Image Library
RTU	Recognisable Taxonomic Unit (Oliver, I. and Beattie, A. J. (1993). A possible method for the rapid assessment of biodiversity. *Conservation Biology*, **7(3):** 562–568)
TARDIS	*Trogoderma* And Related Dermestids Identification Service

7. Plain English website summary

CRC project no:	CRC20137
Project title:	Khapra beetle Diagnostics
Project leader:	Dr J. H. Botha
Project team:	Dr J. H. Botha, Mr R. J. Cunningham, Dr M. Quader and Mr A. Szito
Research outcomes:	1. A national laboratory with expertise in the diagnostics of khapra beetle and related species has been established within DAFWA 2. A trapping program to collect species similar to khapra beetle has been conducted, collecting approximately 10000 specimens from 91 sites around Australia 3. There has been ongoing taxonomic work to understand and better recognise khapra beetle and related species and to provide training and other resources to help with the recognition of this pest 4. A DNA 'fingerprint' type technique has been developed to perhaps allow future identification of khapra beetle using rapid and reliable DNA tests
Research implications:	There has been considerable work done to improve our ability to recognise khapra beetle. Work has been done to study similar species, provide resources to help others identify the species, and develop new tools with the potential to rapidly identify the species. There is a great opportunity for some of these new tools to significantly improve our ability to detect and identify khapra beetle rapidly.
Research publications:	1. Byrne, O., Szito, A., Castalanelli, M., Scanlon, P., and Grimm, M. (2009). Establishment of a national reference laboratory for *Trogoderma* diagnostics. Poster presented at CRC Science Exchange (Diagnostic Platforms) 2. Byrne, O., Szito, A., Scanlon, P., Castalanelli, M., Botha, J., and Grimm, M. (2010). National reference laboratory for *Trogoderma*. Australian Entomological Society's 41st AGM and Scientific Conference 3. Byrne, O., Szito, A., Grimm, M., Chami, M., and Botha, J. (2011). National trapping program for *Trogoderma* and related Dermestids. In *Science Exchange 2011*. Barossa Valley 4. Cunningham, R., Botha, J., Byrne, O., Grimm, M., Quader, M., and Szito, A. (2012). Recognising a wolf in sheep's clothing: detecting Khapra beetle in Australia. Conference abstract CRC National Plant Biosecurity Science Exchange 2012 Swan Valley
Acknowledgements:	There is a *very* long list of people warranting thanks, far too many, to be named here. Hopefully all those who've assisted

	with the project and especially AGI collaborators in the trapping programme, understand our appreciation of their efforts. One person requiring special attention is DAFWA's Mike Grimm who was one of the driving forces behind the project in its early stages. Thanks to CRC/GRDC for their funding and other support with the project and to DAFWA for their institutional support for the project.