PHOTO: CHRIS FREEBAIRN, QDPI&F

The genome of the rust-red flour beetle (*Tribolium castaneum*) has recently been sequenced in the US. Having this additional genetic information will help fast-track genetic and proteomic detection and monitoring tools.

Rapid tests for phosphine resistance

Knowing the extent of a resistance problem is the first step in control

CURRENTLY THE ONLY method for detecting and monitoring insects with resistance to phosphine is to treat insects with a 'discriminating dose' (DD) of phosphine and count the survivors. This method is laborious and slow, as it can take months to breed enough insects from each collection before exposing them to phosphine.

Two research teams are working on developing rapid tests for phosphine resistance. One is using genetic differences and the other protein differences as a method of distinguishing susceptible and resistant insects.

Dr David Schlipalius, of the Queensland Department of Primary Industries and Fisheries (QDPI&F) and the Cooperative Research Centre for National Plant Biosecurity (CRCNPB), has established that two major genes are responsible for higher-level resistance in the lesser grain borer (*Rhyzopertha dominica*). Individually each of these genes is weak, but when present in the same insect they act synergistically to give a much higher resistance.

This high-level resistance can be controlled by appropriate concentrations and exposures to phosphine and is relatively rare because it requires this two-gene resistance mechanism.

DNA markers that are very close to these resistance genes within the lesser grain borer's genome have been found and are being used to target and identify the resistance genes.

A second reference species, the rust-red flour beetle (*Tribolium castaneum*), has been added to the project as the genome of this species has recently been sequenced in the US. Having this additional genetic information will help fast-track this research.

Within the next two years it is hoped that DNA tests for genetic resistance can be incorporated into the national resistance monitoring program. The research team also plans to use the DNA test in ecology projects designed to identify the major factors involved in breeding phosphine resistance in wild populations.

At CSIRO Entomology, Dr Peter Campbell is investigating proteins as a means of rapidly differentiating susceptible and resistant insects. He is also working with lesser grain borer and rust-red flour beetle.

Previous research had suggested that susceptible and resistant lesser grain borer have different levels of the protein arginine kinase. Neither the site of action nor mechanisms of resistance to phosphine are known, but arginine kinase has a function in improving cell respiration by enabling an extra energy store. As phosphine is known to only be toxic to insects that are actively using oxygen, it appeared this protein was worth further investigation.

Dr Campbell repeated the original experiments using a more robust research method and with a wider range of sources of resistant and susceptible lesser grain borer. His study, which involved two years of work, was not able to support the original findings. Nonetheless it was decided to persist for the remaining year of the project with a related approach. Rather than extracting the protein from the whole insect, it was decided to focus on the proteins found in the mitochondria. Mitochondria are found in every cell and play a crucial role in energy conversion and respiration. Various lines of evidence suggest that mitochondria are likely sites of crucial differences between susceptible and resistant insects.

During this year of breeding-up insect populations, grinding and centrifuging to extract the mitochondria and protein analysis, Dr Campbell is optimistic that this research could reveal a lead for the development of a rapid method to identify and distinguish resistant insects.

It is not immediately obvious whether a DNA-based or protein-based test would be the most rapid and portable for use on-site. If the industry is lucky it may have a choice of two methods of rapid phosphine resistance testing. Alternatively, both methods may be required to fully understand how resistance occurs. □

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