GRDC DRIVING AGRONOMY

Double gene drives grain borer resistance to phosphine

Understanding the genetics of insect resistance to chemical fumigants is one of the research strategies for maintaining Australia's 'insect free' grain exports

Lesser grain borer is one of the most serious pests in stored grains in Australia – and very resistant to many insecticides.

BY CATHERINE NORWOOD

The capacity for stored-grain insects to evolve their way around the limited number of environmentally safe fumigants available is a constant challenge for the Australian grains industry, with the potential to jeopardise annual grain exports worth $7 billion.

One of the greatest threats to emerge is the development of resistance to phosphine, the most widely used stored-grain fumigant, in the lesser grain borer (*Rhyzopertha dominica*). Research into the molecular genetics of the species shows some strains that have inherited double resistance genes from both parents are 600 times (or more) resistant to phosphine than insects with none of the genes.

The lesser grain borer is one of nine insect species collected from on-farm and central grain storages identified with varying levels of phosphine resistance. The GRDC's manager for new grain products, Dr Jody Higgins, says the loss of phosphine as an effective fumigant would be a major threat to Australia's reputation as an exporter of clean, insect-free grain.

She says Australia's 'zero tolerance' policy for insect infestations in exports has given the country an edge in the highly competitive international grain market. To help maintain this edge the GRDC, along with major grain companies CBH, GrainCorp and Viterra, is investing in stored-grains research and development through the Cooperative Research Centre (CRC) for National Plant Biosecurity.

Projects underway as part of the CRC's Post-Harvest Integrity Program include:

- molecular analysis of the phosphine resistance in the lesser grain borer;
- new fumigation protocols; and
- a study into the ecology of the lesser grain borer and rust-red flour beetle.

Molecular analysis of the lesser grain borer has found that two genes on separate chromosomes control phosphine resistance. When resistance first appears in a population it tends to be the result of just one of these genes being active, providing just enough resistance to allow an insect to survive low concentrations of phosphine, such as might occur in an unsealed silo. Trouble builds when these initially rare survivors breed and allow both genes to become active in subsequent generations.

Lesser grain borers with high resistance to phosphine were first identified in Bangladesh and India in the early 1990s. Low levels of resistance were found in Australia at the same time and in 1997 highly resistant borers were discovered in storages in Queensland.

In Australia's northern grains region almost every strain of lesser grain borer tested has at least one of the resistance genes. Insects that inherit both genes from both parents are still relatively rare, occurring in only five per cent of insect samples from the north.

A senior lecturer at the School of Mathematical Sciences at Queensland University of Technology, Dr Glenn Fulford, has been modelling the development of resistance in populations of lesser grain borers and says insects with one resistance gene will be 2.5 to 30 times more resistant to phosphine than insects with no resistance genes, depending on where the gene occurs.

Insects with both resistance genes inherited from both parents are at least 250 times, and possibly upwards of 600 times, more resistant than insects with no resistance genes. Dr Fulford says the double-gene nature of resistance in borers has delayed its development and given the industry some breathing space to develop counter-measures. But it also significantly increases the level of resistance where it occurs.

The leader of the CRC's Post-Harvest Integrity Program, Dr Pat Collins, from Queensland's Department of Employment, Economic Development and Innovation (DEEDI), says the growing resistance to phosphine puts Australian grain traders in a precarious position.

"We harvest and store our grain through the hottest part of the year, when insects are most active. Other grain-growing countries like the US and Canada don't have this problem to the same extent because their grain goes into storage in cooler weather."

The resistance issue stems from the worldwide popularity of phosphine, which is cheap and effective, easy to apply and environmentally benign. It can be used for multiple commodities and is effective on a wide range of insects. There is also no readily available, comparable alternative.

More than 70 per cent of all grain in Australia is treated with phosphine; it is the chemical most in contact with insects, creating the greatest chance for insects to develop resistance. This begins when some insects start to survive treatment, often because of inadequate procedures.

Dr Collins says managing phosphine resistance has also been made more difficult by the deregulation of the grains industry in Australia. There are new players entering the grain marketing and export business and there is a substantial rise in on-farm grain storage. It means more people need to be informed about the issue and skilled in fumigation procedures.

Viterra's grain hygiene manager Greg Hopkins says the company's investment in the CRC is to support investigations into ways to extend the life of phosphine, to slow the spread of resistance and find alternative products.

"Without phosphine the cost of protecting grain could easily increase by more than $100 million a year, or from $1 a tonne per treatment to $5. There has already been an incremental loss of effectiveness, with more frequent and longer fumigations required to kill insects. It will affect the bottom line of every grower," Mr Hopkins says.

New fumigation protocols are part of the responses developed through the Post-Harvest Integrity Program, directed by DEEDI's Dr Manoj Nayak. A new same-day test provides grain handlers with rapid confirmation of insect resistance, allowing them to adjust fumigation strategies before moving grain.

Lesser grain borer resistance is currently being controlled as a result of access to the rapid testing and revised fumigation protocols, which are proving effective at killing even the most strongly resistant strains of the species found to date. Where resistant lesser grain borers are identified, new protocols apply phosphine at a rate of 720 parts per million over seven days. This compares to phosphine applied at 720ppm for one day on non-resistant insects.

Dr Nayak says in recent years high levels of resistance have also emerged in flat grain beetles, which had not previously been

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considered a serious problem in stored grain. Where resistance is identified, new fumigation protocols for flat grain beetles apply phosphine at 720ppm over 24 days, or 360ppm over 30 days, to effectively kill all insects.

Dr Nayak says 65 different strains of the original flat grain beetle species, *Cryptolestes ferrugineus*, have been identified with high resistance from 54 central grain storages and two farm storages during the past three years.

While the GRDC is investing in a range of research to better understand how phosphine resistance is developing and strategies to counter it, Dr Higgins says quality on-farm storage is the first line of defence in maintaining the effectiveness of phosphine.

Best practice guidelines for on-farm grain storage and fumigation include:

- using silos that can be sealed to maintain effective concentrations of phosphine during fumigations – check with the manufacturer that it meets the Australian Standard for sealable silos. Fumigating with phosphine is only fully effective in a storage that is gas-tight;
- using silos with aeration capacity to cool grain and reduce breeding activity;
- fumigating grain during warmer months when insects are more active; and
- cleaning grain handling and storage equipment and disposing of or treating old, infested grain. □

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