CRC PLANTbiosecurity

MEDIA RELEASE

For immediate release: 30 November 2009

Biosecurity defends key rural industry

Australian researchers are studying genetic codes to learn how insects like the lesser grain borer are developing resistance to the world's most widely used grain fumigant – phosphine.

By Catherine Norwood

With the harvest safely stored, grain growers often take a moment to relax; but not so the scientists involved in a national research effort to keep stored grain safe from feasting insects and to maintain Australia's hard-earned reputation as a supplier of clean, pest-free grain.

Stored grain insects are a constant threat to grain exports – worth some $7 billion each year to the national economy.

Australia offers overseas grain buyers a 'zero tolerance' benchmark for insect infestation and this is a key element in Australia's international competitiveness.

However, the capacity for insects to evolve their way around the limited number of environmentally safe fumigants available poses a constant challenge – one that is now being tackled by the Cooperative Research Centre (CRC) for National Plant Biosecurity.

The latest research is turning to the insects themselves and the genetic coding that allows them to develop resistance, especially resistance to the principle fumigant, phosphine.

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. Fortunately this is still rare enough to give the industry some breathing space in which to learn more about the genetics that drive phosphine resistance, and through this knowledge develop new counter measures.

Varying levels of phosphine resistance have been indentified in nine insect types collected from on-farm and central grain storages around Australia. The most serious threats come from the lesser grain borer (*Rhyzopertha dominica*) and the flat grain beetle (*Cryptolestes ferrugineus*), both of which have shown high levels of resistance.

Leader of the CRC's Post-Harvest Integrity Research program, Dr Pat Collins from Queensland Primary Industries and Fisheries 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."

Dr Collins says the Australian industry's zero tolerance for live insects in its export grain shipments has given the country an advantage in highly competitive international grain market. But rising levels of insect resistance to phosphine have the potential to jeopardise these markets. While Australian wheat deliveries have not been rejected at international ports because of live insect contamination, heavy penalties or discounts can be levied if grain needs to be re-treated on arrival.

The resistance issue stems from the worldwide popularity of phosphine. Phosphine is cheap and effective, easy to apply and is 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.

That's why more than 70 per cent of all grain is treated with phosphine – which means it is the chemical most in contact with insects, thus 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.

The Grains Research and Development Corporation and Plant Health Australia have been members of the CRC for National Plant Biosecurity since its inception in 2005, and three years ago the emerging phosphine issue provided a catalyst for the major grain companies Co-operative Bulk Handling Limited, GrainCorp and ABB Grain Ltd to join the CRC.

Quality and technical services manager for ABB Grain in Australia, Geoff Masters 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 to $5 a tonne per treatment. 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 Masters says.

Lesser grain borer resistance

Research on the lesser grain borer is the most advanced, as widespread high resistance was 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.

Molecular analysis has found that two genes on separate chromosomes control phosphine resistance in lesser grain borers, each of which produces a relatively weak resistance. The carrier of a single resistance gene is known as a heterozygote; a carrier of both resistance genes is a homozygote. When both genes are present it produces a higher level of resistance. However, resistance is also close to recessive and strong resistance is only found when an insect carries both copies of both genes from its parents.

When resistance genes first appear in a population they are rare and resistant individuals carry only one copy, but may have enough resistance to survive low doses of phosphine. These heterozygotes become relatively more common and breed with others, producing homozygous resistant insects. Further fumigations continue to purify the insect population, producing almost entirely resistant strains.

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

Senior lecturer with 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.

Homozygous insects with both resistance genes are at least 250 times and possibly upwards of 600 times more resistant than insects with no resistance genes. Dr Fulford says the homozygous nature of high resistance in borers has delayed its development and given the industry some breathing space to respond to the threat. But the double gene also significantly increases the level of resistance where it occurs.

It is not known yet whether the development of resistance in the flat grain beetle follows the same pattern as that of the lesser grain borer.

New fumigation protocols

New fumigation protocols have been developed for lesser grain borers and other insects through a CRC project directed by Dr Manoj Nayak from Queensland Primary Industries and Fisheries. A same-day test has been developed to provide grain handlers with rapid confirmation of insect resistance, allowing them to adjust fumigation strategies before moving grain. Regional monitoring programs collect insect samples from almost 400 different sites each year including farm storages, grain handlers and central storages. Another 300 or so insect samples are sent for 'tactical testing' after being collected alive from fumigated grain storages.

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. These protocols apply phosphine at a rate of 720ppm over seven days. But Dr Nayak has found that highly resistant flat grain beetles require phosphine application 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. There are also four different species of *Cryptolestes* found in Australian grain storages and varying levels of resistance are suspected in the other three species, although this is still being confirmed.

He says it would be highly unusual if the different species all developed resistance at the same rate, but he is not ruling it out. However, it complicates the development of new fumigation protocols because additional testing will be required to characterise resistance in each species to make sure that protocols developed for *Cryptolestes ferrugineus* are adequate to control other species.

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High-resolution images of the flat grain beetle, and lesser grain borer are available to accompany this media release from www.crcplantbiosecurity.com.au/news/media-releases