AHRI **insight...**

World's first metabolism based glyphosate resistance

By Peter Newman

WINE casks, plastic bank notes, WiFi, the refrigerator, lawn mower and the humble ute (pickup truck) are all Australian, world first inventions. Which of these makes the biggest difference to your life? As long as it's not the wine cask you're doing just fine!

We now have a world first in herbicide resistance.

Glyphosate has been such a successful herbicide largely because plants can't metabolise it. Metabolism of herbicides means that the plant breaks the molecule into pieces before it can get to the enzyme that it's trying to bind to, rendering it ineffective.

In 2011, then Ord River agronomist, Andrew Cripps, was approached by a farmer asking why his Barnyard grass had survived glyphosate. Dr. Todd Gaines and Prof. Steve Powles visited the northern irrigation region, took samples, and confirmed glyphosate resistance. During this research they noticed that the level of resistance was influenced by temperature, leading them to conclude that the mechanism could be metabolism based.

Further research by visiting Chinese researcher to AHRI, Dr. Pan Lang under the watchful eye of Qin Yu, concluded that they were looking at the world's first case of metabolism based resistance to glyphosate. The culprit? I know it's on the tip of your tongue! That's right, it was a member of the super family of enzymes, Aldo-keto reductase.

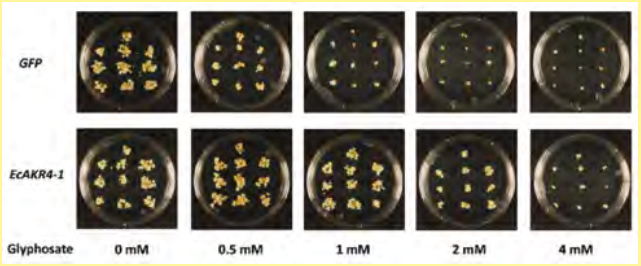
Awnless Barnyard Grass (BYG) *Echinochloa colona* is a very successful grass weed in tropical Australia. It can germinate at all times of year with its main germination and growth occurring in the wet season.

Awnless barnyard grass. (PHOTO: M Widderick)

What led to this resistance?

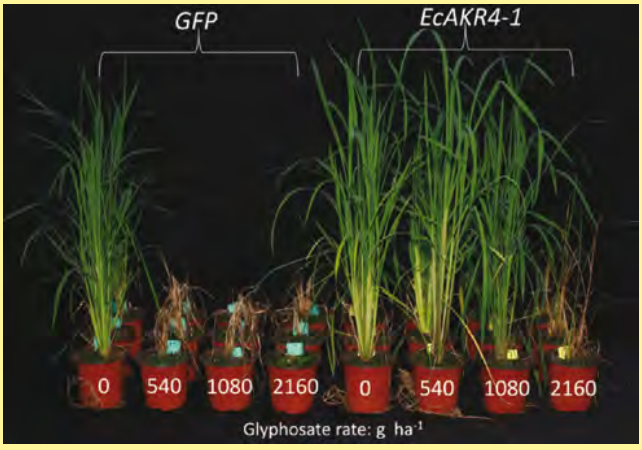
This population of Barnyard grass was found in one of the more recently cleared areas of the Ord river irrigation area. This is a drip irrigation area so cultivation is not an option for weed control. Horticultural crops such as melons and pumpkins were commonly grown and the farmers like to keep the field weed free during the wet season so that crops can be planted as soon as the wet season finishes to take advantage of early market opportunities. The property was aerially sprayed with five to six litres per hectare glyphosate, five to six times per wet season for about 12 to 15 years in a row. This was the first Barnyard grass

FIGURE 1: Picture of rice calli growing on agar infused with different concentrations of glyphosate



GFP = control. EcAKR4-1 is rice calli with the over expressed AKR gene.

FIGURE 2: Picture of transgenic rice sprayed with different rates of glyphosate



GFP = control. EcAKR4-1 is rice calli with the over expressed AKR gene.

population to be confirmed resistant to glyphosate in the region and several more have since followed.

Glyphosate metabolism

While there is some evidence of glyphosate metabolism in some plant species (notably legumes), and metabolism by soil microbes is common, there is no evidence of a weed species evolving resistance to glyphosate by metabolism.

Temperature

The first clue that metabolism was at play was the response to temperature. When resistant Barnyard grass was grown at 35 to 30 °C, all plants survived 540 gai per hectare glyphosate but only 70 per cent survived when plants were grown at 25° to 20°C.

Genetic testing

The researchers used a technique called RNA-seq to look for which enzyme was involved in this metabolic resistance. Aldo-keto reductase (AKR) enzymes are from a super family, so it can be like finding a needle in a haystack. But they did find that one particular (AKR) was over expressed, allowing the plant to make lots of this enzyme. BINGO!

Rice calli

But they couldn't stop there. They needed to be 100 per cent sure that this AKR enzyme was causing the resistance. The use of rice calli in molecular biology research is relatively new.

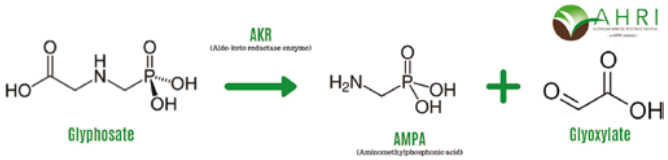
What is rice calli I hear you ask? Think stem cells. Calli is rice tissue culture that is grown on agar. This calli is like stem cells that you may have heard of that can differentiate into any part of a rice plant. The researchers expressed the AKR gene in the rice calli and then grew that calli tissue culture on agar infused with glyphosate. BINGO! They confirmed that the over expression of this AKR gene was responsible for the resistance.

One last step, to be 100 per cent sure

Rice calli is great science, but there's nothing quite like growing a resistant plant and spraying it. The researchers took one final step to produce transgenic rice plants with the over-expressed AKR gene and voila, the transgenic rice plants were resistant to glyphosate whereas the controls were normally susceptible.

Metabolism

How does it all work? In simple terms, the glyphosate molecule is broken in half by the AKR enzyme. The bits that it's broken into are called the metabolites. One of these, AMPA, still has some herbicidal activity, so the plant has more work to do to break down AMPA before it's in the clear.



Conclusion

This research goes to show that there's always another mechanism! In the past we have seen several glyphosate resistance mechanisms, but never metabolism based resistance. This is a world first, and with new research techniques being developed to identify these mechanisms we're sure it won't be the last.