About This Issue

Welcome to another IRAC eConnection newsletter. As always, we try to bring you interesting and informative articles about the work of IRAC and keep you updated on developing insecticide resistance issues around the world.

In this issue we report on the IRAC-US sponsored symposium on the use of insecticide mixtures, particularly in relation to resistance management; we include an overview on research on the cytochrome P450 mechanism; there is an update on activities and resources being developed to combat resistance to the tomato leafminer, *Tuta absoluta* and a review of articles and reports on insecticide resistance appearing in publications over the last few months.

Remember, if you have any news or resistance topics of interest, please let us know so that we can inform others in the IRAC Network. We hope you enjoy the issue.


**Insecticide Mixtures: IRM, Science, Scope, Solutions and Rationale**

The symposium provided a comprehensive overview of the use and impact of pesticide mixtures from several view points which included researchers, crop consultants, extension and industry representatives.

The use of mixtures, the simultaneous exposure of a pest to two or more active ingredients, is a complex topic. While most mixtures are not primarily used for IRM, they do have an impact on the susceptibility of pest populations. The large number of possible components, the characteristics of each, possible interactions of the components, effects on pest species and other factors combine to make the number of possible combinations and interactions extremely large. Consequently, every mixture requires considerable evaluation before it is made and applied. When using single active ingredient products, pre-mixtures or tank-mixtures it is important to always follow label recommendations.

Member companies within IRAC have been developing a position statement on the use of mixtures for insecticide resistance management. A preliminary draft was presented during the IRAC US Symposium and it is hoped that this will be finalised at the IRAC International Spring Meeting in Indianapolis in March. A full report on the IRAC-US symposium is being prepared and will be published in due course.
Perspectives on Resistance Management - Focus on P450

Insecticide resistance is principally based on two important mechanisms in a selected strain or population of invertebrate pests, i.e. target-site modification and enhanced metabolic detoxification or both. Additional mechanisms of resistance include for example delayed penetration, sequestration or elimination via drug efflux pumps. Target-site resistance is due to mutations in the protein addressed by the insecticide, and metabolic resistance is conferred by a number of different enzymatically driven detoxification mechanisms.

One of the most important families of detoxification enzymes are the heme-containing cytochrome P450’s (EC 1.14.14.1), and CYP genes coding these enzymes constitute one of the largest known family of genes. The number of CYP genes in yet sequenced insect genomes range from 36 (Pediculus humanus) to 170 (Culex quinquefasciatus). Cytochrome P450’s catalyze numerous reactions which could result in toxic, active and inactive metabolites of a vast range of organic compounds including xenobiotics such as insecticides.

Hydroxylation is considered to be the chief reaction in Phase I metabolism of xenobiotics (RH) and described by the general reaction equation: \( RH + O_2 + NADPH_2 \rightarrow R-OH + H_2O + NADP \) (one atom of oxygen enters R-OH and one atom enters water, and due to the dual fate of oxygen, monooxygenases are also called mixed-function oxidases). The metabolic detoxification of insecticides is also mainly conferred by P450’s, which are up-regulated and over-expressed in resistant strains of many of the world’s most destructive crop-, stored product- and public health pests. In various insect and mite pest species, cytochrome P450-mediated oxidation of insecticides is the key mechanism of resistance and cross-resistance.

The suppression of resistance by synergists such as piperonyl butoxide (which irreversibly inactivates P450’s) in a whole organism bioassay usually provides a first line of evidence on P450 involvement in resistance. During the last few years the number of complete arthropod CYPomes increased considerably due to a steady increase of sequenced genomes, including those from relevant pest arthropods (1). In the last few years several P450’s showing constitutive over-expression in resistant strains of pest insects were identified by microarray analysis. Subsequent cloning of the coding sequence and functional expression in combination with cytochrome P450-reductase often confirmed their direct involvement in the detoxification of insecticides. This has recently been shown to work especially for a number of different insect P450’s of the CYP6-family, e.g. CYP6CM1 of neonicotinoid-resistant strains of Bemisia tabaci (2), CYP6BQ9 in pyrethroid-resistant Tribolium castaneum (3), and CYP6M2 in pyrethroid-resistant Anopheles gambiae (4); just to name a few. The current knowledge on insect P450’s was recently reviewed in (5) and provides excellent further reading.

References
1. Feyereisen R. (2011) Arthropod CYPomes illustrate the tempo and mode in P450 evolution. BBA 1814, 19
Report on the *Tuta absoluta* Meeting in Agadir

From 16-18 November 2011, Agadir, Morocco was the site of the joint EPPO/IOBC/FAO/NEPPO symposium on management of *Tuta absoluta*. Two hundred participants from more than 30 European and Mediterranean Countries as well as from the Middle East met for three days of knowledge exchange and strategies to fight this dangerous invasive pest.

The symposium provided a common forum for researchers, regulatory authorities, experts from extension services or advisory bodies, and the crop protection industry, to share their knowledge in pest biology, phytosanitary measures, control measures, particularly biological control and insecticide resistance; to identify gaps in knowledge and research needs in order to avoid duplication of work.

IRAC, as collaboration partner of this symposium, contributed actively in the Session “Control Strategies: Chemical Control and Mating Disruption” with a presentation “General Introduction on the Insecticide Resistance Action Committee”, which included information on the activities of the Methods Working Group (IRAC Method No: 022) and the Lepidopteran Working Group on *Tuta absoluta*. Besides this presentation, IRAC was also represented during the whole symposium with a stand displaying posters on the international activities of IRAC and specifically, recommended IRM strategies for *Tuta absoluta*. Copies of the recently published *Tuta absoluta* brochure and MoA brochure were handed out and there was also great interest in IRAC Method No: 022 with the video being shown and print-outs distributed.

Aside from the symposium a new IRAC Diamide Country Group (Morocco) was formed under the guidance of Andrea Bassi, Dupont, who sits on the both the IRAC International Diamide and Lepidoptera Working Groups.

All in all there were lots of fruitful discussions and follow-ups are ongoing.
IRAC poster on *Tuta absoluta* Poster

This new poster from IRAC provides background to the development of the pest, describes the symptoms, damage and life cycle and then gives detailed guidelines on resistance management and integrated control strategies to prevent or reduce the spread of resistance of this invasive pest.

IRAC *Tuta absoluta* Susceptibility Test Method Video

The IRAC Methods Team have started developing videos, posted on YouTube with links from the IRAC website, to complement the existing Method Series documents. This is a big advantage for researcher to see in practice how the method should be carried out rather than just reading a description. The *Tuta absoluta* video was the first IRAC method video (See: [http://www.youtube.com/watch?v=PSE_MwIAV0s](http://www.youtube.com/watch?v=PSE_MwIAV0s)) but this has since been followed with a video on aphids using *Myzus persicae* as the example species (See: [http://www.youtube.com/watch?v=jE359LnpBnI&feature=related](http://www.youtube.com/watch?v=jE359LnpBnI&feature=related)).
Spotlight on recent Insecticide Resistance Articles in Publications

‘Whitefly make for adult-only reading’
The development of resistance to neonicotinoid insecticides continues to be a major area of interest for insecticide resistance researchers globally. Although whiteflies were one of the first recorded species to develop resistance to this insecticide chemistry, new findings continue to be made. Studies have been conducted to explain the age-specific resistance observed in the tobacco whitefly (Bemisia tabaci) [1]. It has recently been observed that adult whitefly express high levels of resistance, but nymph stages of ‘neonicotinoid resistant’ B. tabaci can still be effectively controlled with the same chemistry [2]. This is explained as by an adult-only up-regulation of the CYP6CM1 gene, which is thought to be the major factor in neonicotinoid resistance in this species. Similarly resistance to neonicotinoids in the glasshouse whitefly (Trialeurodes vaporariorum) has been well known for some time, but is only recently been well documented [3]. Work also continues to characterise the reported neonicotinoid resistance developments in Asian citrus psyllid (Diaphorina citri) [4], cotton aphid (Aphis gossypii) [5,6] and the green peach aphid (Myzus persicae) [7].

‘Mites increase Euro-Chinese relations’
In 2010 field evolved resistance was reported by Chinese researchers in citrus red mite (Panonychus citri) and this is followed by a report of regionally evolved field-resistance to spiromelenofen in their European cousins, the European red mite (Panonychus ulmi), although as with the neonicotinoid resistant whitefly, resistance seems to be age-specific [8].

‘The usual suspects strike again’
The beet armyworm (Spodoptera exigua), diamondback moth (Plutella xylostella) and the cotton bollworm (Helicoverpa armigera) continue to demonstrate their ability to evolve resistance to new chemical control methods, with reports of methoxyfenozide/tebufenozide resistant H. armigera [9] and indoxacarb resistant S. exigua [10] in China. Emamectin benzoate resistant P. xylostella is also recorded in India [11].

‘Rootworms and Bollworms’
Field evolved resistance to transgenic crops expressing Bt toxins has been one of the major discussion points during the second half of 2011. The link between the increased survival of western corn rootworm larvae (Diabrotica virgifera) to the Cry3Bb1 toxin and damaged crops in Iowa, USA [12] continues to be major discussion point globally. As do the reports of some field populations of the pink bollworm (Pectinophora gossypiella) developing resistance to Cry1Ac fed to the insects as a seed powder [13].

References:
9. Liu J, Dong LX, Tan XW, Fan XL, Rui CH, Cross resistance to 12 insecticides in methoxyfenozide-resistant populations of Helicoverpa armigera
IRAC News Snippets

★ IRAC International has now set up a new working group focussed on resistance management in Coleoptera which includes the team working on pollen beetles and other pests of oilseed rape. Conference calls with the new group were held in August and December 2011 to set objectives for the team in the 2012. IRAC Members interested in joining the team should contact Alan Porter via the website or at the address below.

★ A monitoring programme investigating reports of resistance in the green peach aphid (*Myzus persicae*) was carried out by IRAC in conjunction with Rothamsted Research, UK. Samples from populations in Southern France, Spain and Italy were sent to Rothamsted for analysis and a meeting was held this month in Barcelona to discuss the results. Look out in future eConnections for more details.

★ IRAC is in the process of developing new posters covering available modes of action for the control of Coleoptera and insecticide resistance management strategies in citrus psyllid and *Spodoptera exigua*.

★ The IRAC website continues to be very popular. User statistics from the website for 2011 show that visits are up by 20% and number of page views up by 9% compared to 2010.

Conferences & Symposia

★ 7th Intl. IPM Symposium, Memphis, Tennessee, March 27-29, 2012
★ 47th IRAC International Meeting, Indianapolis, USA, March 27-30, 2012
★ XXIV Intl. Congress of Entomology, Daegu, S. Korea, August 19-25, 2012
★ 60th ESA Meeting, Knoxville, Tennessee, November 11-14, 2012
★ 61st ASTMH Meeting, Atlanta, Georgia, November 11-15, 2012

Feedback
The eConnection is prepared by the IRAC International Communication & Education Working Group and supported by the 15 member companies of the IRAC Executive. If you have information for inclusion in the next issue of eConnection or feedback on this issue please email aporter@intraspin.com

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