Investigation of abamectin resistance following expression of *Tetranychus urticae* CYP392A16 in *Drosophila*

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Agricultural production protection largely relies on the control of pest populations with the use of insecticides. However, pests display an intriguing ability to develop resistance through several mechanisms that may include target-site alterations and/or overexpression of detoxification genes.

Abamectin resistance in the two spotted spider mite *Tetranychus urticae* has been associated with target-site mutations in the Glutamate-gated chloride channel (*GluCl*) [1,2] as well as with overexpression of certain cytochrome P450s, like CYP392A16 which has been shown to metabolize abamectin *in vitro* [3] (Figure 1).

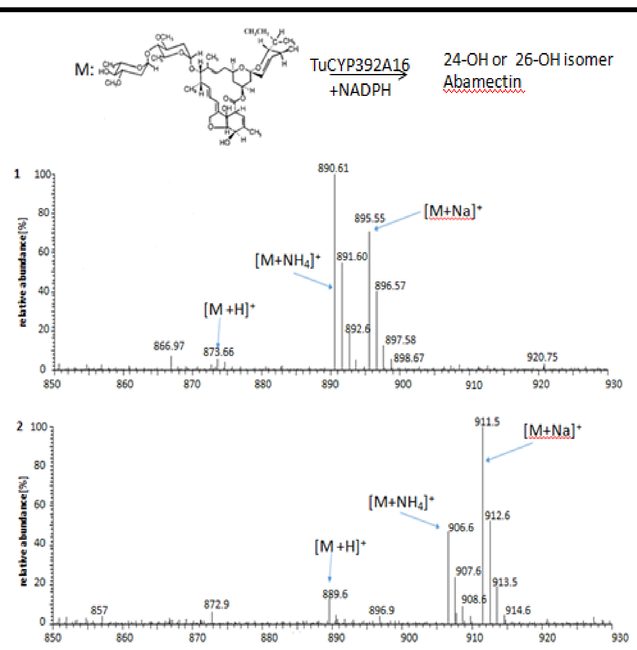

Figure 1. Analysis of abamectin metabolites *in vitro* following incubation with *T. urticae* CYP392A16 expressed in bacteria [3].

In order to validate the role of CYP392A16 *in vivo* we performed heterologous GAL4/UAS overexpression of the protein (together with the associated TuCPR) in transgenic *Drosophila*, with the use of a Gal4 driver driving expression in detox-related tissues. (Figure 2).

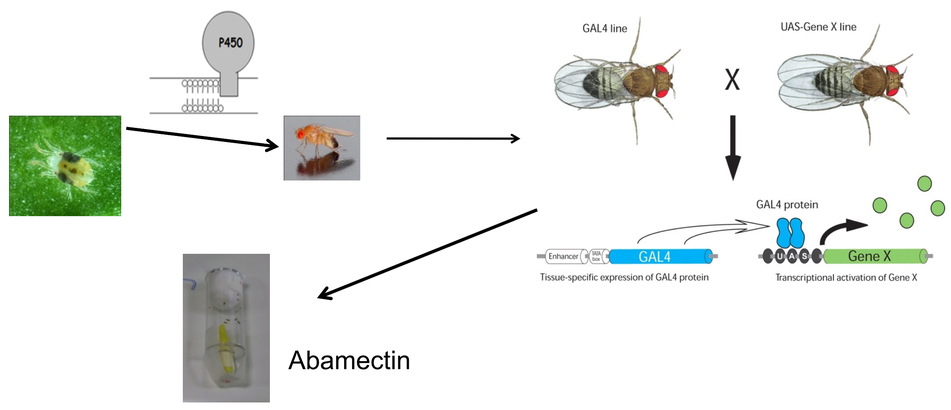

Figure 2. The GAL4/UAS expression system is employed for tissue-specific transgenic expression of the TuCYP392A16 (along with relevant TuCPR) in transgenic fly strains. Transgenic expression confers modest levels of resistance to abamectin as shown by tocixity bioassays (Table 1).

We performed a series of toxicity bioassays comparing transgenic strains versus susceptible controls in order to investigate the ability of CYP392A16 to confer abamectin resistance *in vivo* (Table 1).

Table 1. Bioassay results comparing a *Drosophila* strain expressing TuCYP392A16 and TuCPR under the HR-GAL4 driver, against a strain bearing the same transgenic alleles and genetic background but no GAL4 driver. Moderate resistance levels are achieved in adult feeding toxicity bioassays.

Transgenic Lines	Regression parameters		
	LC_{50} (mg/L)	X^2	RR
	95% CI		95% CI
UAS-CYP392A16; UAS-TuCPR x HR-GAL4	53 (47.4-57.6)	14	1.69 (1.4 – 2.06)
UAS-CYP392A16; UAS-TuCPR x w^{1118}	31.2 (14.7-37.6)	30.9	-

We are in the process of generating relevant *Drosophila GluCl* mutants with the use of CRISPR/Cas9 (Figure 3) aiming to validate candidate traget-site resistance mutations introduced into a susceptible genetic background.

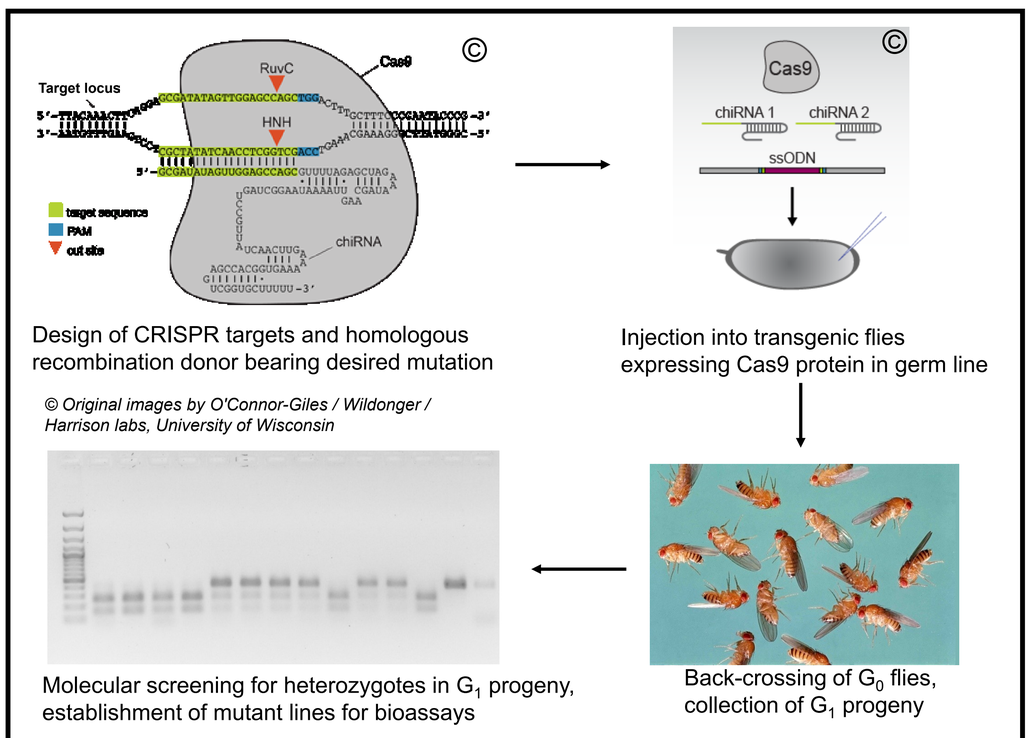

Figure 3. CRISPR/Cas9 genome modification system can be employed for generation of candidate target-site mutations [4, 5]. We are in the process of generating mutations of interest in the *Drosophila* ortholog of the *T. urticae GluCl* gene. The mutations (G→D/E) are generated at position 312 of the *Drosophila GluCl,* which is equivalent to either the G323D [1] or the G326E [2] mutations identified in certain *T. urticae* resistant strains.

Furthermore, we plan to combine *Drosophila GluCl* mutants with the existing transgenic strains overexpressing TuCYP392A16 in order to investigate confounding abamectin resistance mechanisms in a controllable genetic background, taking advantage to this end of the unique available genetic toolkit and standard *Drosophila* genetics.

Despite certain limitations, this approach may significantly enhance our ability to investigate complex insecticide resistance phenotypes by engineering *Drosophila* lines bearing several resistance genes and/or mutations simultaneously.

Acknowledgements

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References

[1] Kwon et al. (2010) *Insect Mol Biol* 19: 583-591
[2] Dermauw et al. (2012) *Insect Biochem Mol Biol* 42: 455-465
[3] Riga et al. (2014) *Insect Biochem Mol Biol* 46: 43-53
[4] Douris et al. (2016) *PNAS* 113: 14692-14697
[5] Douris et al. (2017) *Insect Biochem Mol Biol* 87: 127-135