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INTRODUCTION

The availability of effective pesticides is essential for human health, prosperity and food security. Both, agricultural production and the control of vectors carrying life-threatening diseases depend on effective pest control and this is mostly dependant on pesticide usage. The synthetic pyrethroids, photostable derivatives of the natural pyrethrum compounds from *Chrysanthemum* flowers, are frequently used to control tick/mite infestations in agricultural, veterinary and domestic (home/garden) settings and command a large share of the pesticide market.

The voltage-gated sodium (Na) channel of nerve membranes is known to be the principal target of pyrethroid action and a number of point mutations in the domain II and III helices of the channel protein have been identified that confer resistance in a range of insect pests (Fig. 1). Recent studies have also demonstrated that target-based resistance is on the increase in important tick and mite species, so a clearer understanding of the nature of the interactions of pyrethroid acaricides with their target is becoming an urgent priority in the battle to control acarine pests. In this project we characterized the interactions of pyrethroids with tick/mite Na channels at the molecular level, identified novel resistance mutations in field populations of mites and developed a rapid, high throughput DNA-based assay for diagnosing the presence/absence of resistance mutations in field populations of *Varroa destructor*.

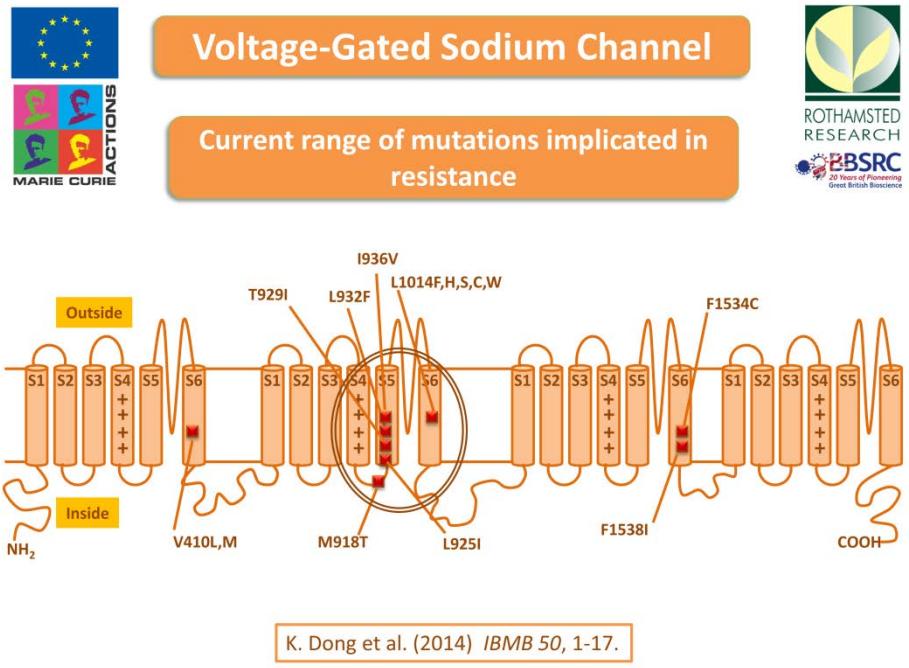

Figure 1. Schematic diagram of the Voltage Gated Sodium Channel showing mutations associated with resistance to pyrethroids in arthropods species. Data from: Dong K, Du Y, Rinkevich F, Nomura Y, Xu P, et al. (2014) Molecular biology of insect sodium channels and pyrethroid resistance. Insect Biochem Mol Biol 50C: 1-17.

SUMMARY OF RESULTS

"In silico" analysis of Voltage Gated Sodium Channels (VGSC) sequences available in databases revealed several very important differences between species in regions of the channel directly in contact with the pyrethroids i.e. the linker between transmembrane segments 4 and 5 of Domain II (DII S4 and S5), the DII S5 helix as well as the DII P-loop and the DIII S6 helix. According to our analysis, the differences found may explain the well-known differences in the susceptibility of the

species to pyrethroids. They also highlight other species-specific polymorphism that can potentially be exploited to design more specific insecticides needed for control of target species but without effects on non-targets. For example, control of varroa mites on honeybees or two spotted spider mites in greenhouses where predatory mites are used as biological control agents.
Given the importance of predatory mites for biological control programs, available VGSC sequences from acari were compared and used to design degenerate primers to PCR amplify relevant regions of the channel from species with no sequence information available. Our results revealed that in two of the predatory mite species tested, *P. persimilis* and *A. swirskii*, there were polymorphisms in highly conserved residues in the binding site of pyrethroids. Since most of these species of predatory mites are already in use in many countries as part of biological control programs, our findings are of great importance for pest control in the context of Integrated Pest Management strategies. If these mutations do confer resistance to pyrethroids in *P. persimilis* and *A. swirskii*, they will be good candidates for strategies using pyrethroids to control pest species but leaving the predatory mites unaffected.

The infestation of Western honey bees (Apis mellifera) by the ectoparasitic mite *Varroa destructor* has been an important factor contributing to the increasing losses of colonies reported in many European countries. The control of this mite has relied heavily on chemical interventions, with certain synthetic pyrethroids being among the most useful products for selective and effective control of the parasite without harming the bees on which they feed. Unfortunately, the widespread and intensive use of these compounds, as with most pesticides, has now led to selection for resistance nearly worldwide.

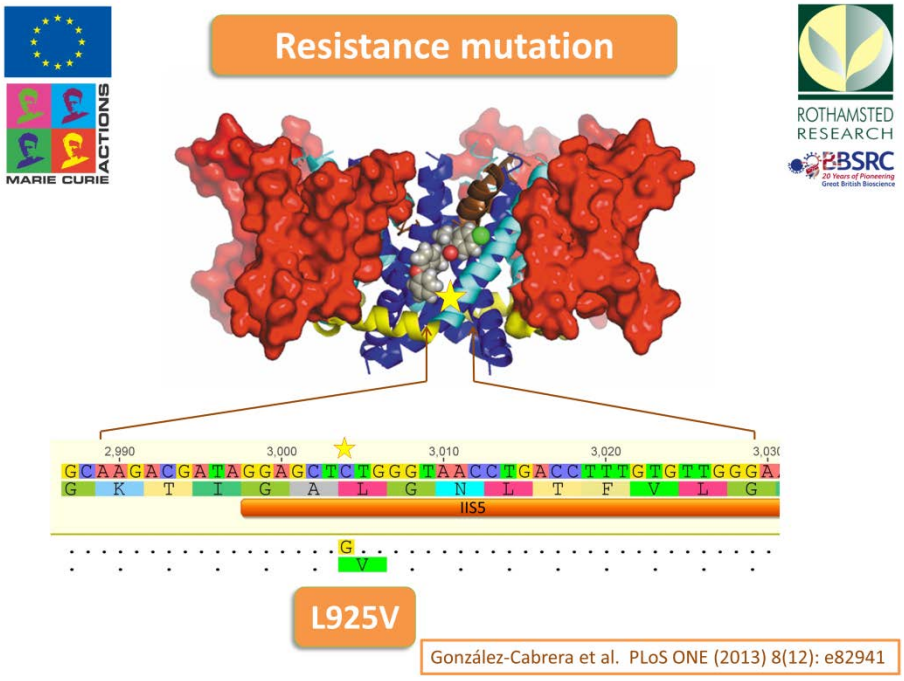

Figure 2. Fragment of *Varroa destructor* sodium channel gene and position of the mutation L925V in the binding site of pyrethroids. The model of the channel has been taken from: O'Reilly AO, Khambay BPS, Williamson MS, Field LM, Wallace BA, et al. (2006) Modelling insecticide-binding sites in the voltage-gated sodium channel. Biochemical Journal 396: 255-263.

Our results evidenced that a novel amino acid substitution, L925V, is associated with the resistance to pyrethroids in multiple populations around Europe. This mutation is located in a known hot spot for resistance within the domain IIS5 helix of the VGSC protein; a region that has also been proposed to form part of the pyrethroid binding site (Fig. 2). The electrophysiological analysis of mutated VGSC

using two electrodes voltage clamp showed clear evidence supporting that L925V is indeed conferring high levels of resistance to pyrethroids. Furthermore we developed a high throughput diagnostic assay capable of detecting the mutation in individual mites that confirmed that L925V is present in samples collected in the UK but also in Belgium, France, Germany, Italy and Spain. Moreover, as previously found in our earlier analysis, there is a strong correlation between the presence of the mutation and the recent use of pyrethroids in Varroa management strategies (Fig. 3). The advantage of DNA-based assays for mutation detection and resistance monitoring are their relatively low cost, speed, and ability to test poor quality and even dead samples. Indeed, we have found that this assay is extremely robust and capable of accurately genotyping individual dead mites collected from hives and stored at ambient temperatures over several days. This in turn makes it feasible to assess the status of individual hives for the presence and/or frequency of the mutation before deciding whether treatment with pyrethroids is likely to be successful.

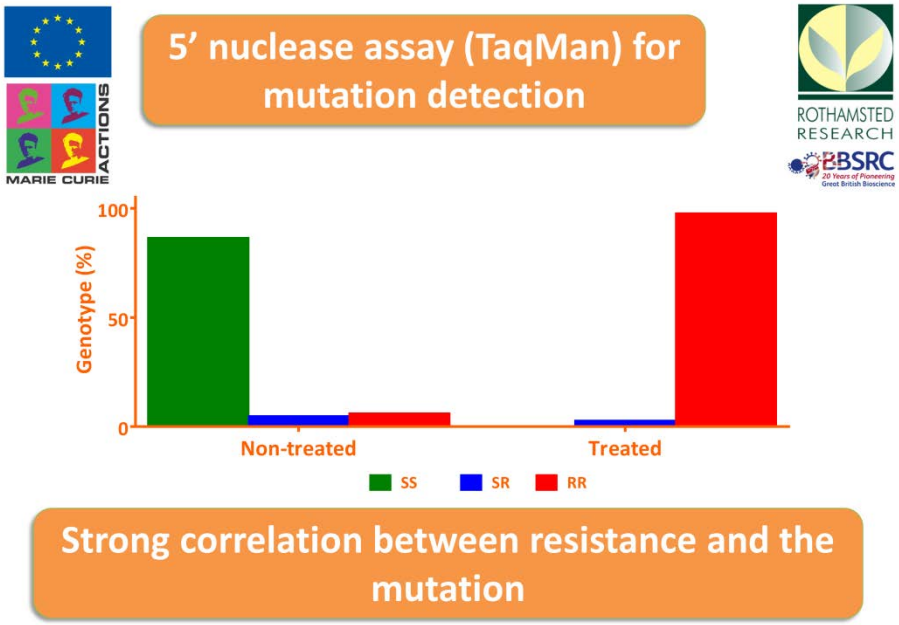

Figure 3. Allelic frequencies of the mutation L925V determined using Real-time TaqMan assays in *Varroa destructor* samples collected from treated and non-treated colonies. RR = resistant homozygote, SR = heterozygote, SS = wild-type