

How does the new acaricide 1-allyloxy-4-propoxybenzene work? Studies on the mode of action using proteomics and fluorescence microscopy

Erika Plettner¹, Laura Chapman¹, Niloufar Choubdar¹, Osei Fordwour², Xinyi Feng¹, Leonard Foster²

¹Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada

²Department of Biochemistry and Molecular Biology, The University of British Columbia, Vancouver, BC, Canada

The compound 1-allyloxy-4-propoxybenzene, coded 3c(3,6), causes paralysis and death of the mite *Varroa destructor*. We addressed the mode of action of compound 3c(3,6) by exploring the proteins the compound binds to and their location in the mites. The studies began with the preparation of probes that have the structure of 3c(3,6) at one end and either a bead for protein capture or a fluorescent dye for imaging of tissues, connected through a flexible linker. To capture proteins, we prepared extracts of soluble and membrane proteins, and incubated them with the beads modified with 3c(3,6). As negative controls we used beads modified with a structure that resembles 3c(3,6) but we knew from structure-activity studies not to be paralytic to the mites, as well as beads modified with the linker chain only. After washing the various beads, the proteins bound to them were analyzed by proteolysis, followed by liquid chromatography-mass spectrometry (LC-MS). Comparison of the peptides detected in the 3c(3,6) treatment vs. the negative controls enabled us to determine which proteins bind the active compound. To image the binding sites of 3c(3,6) in the mites and bees, we incubated tissues with the fluorescent probes with 3c(3,6) or the inactive structure or the linker, the latter two being the negative controls. Tissues were embedded, cryosectioned and imaged on a confocal microscope.

Soluble proteins we found include lipid-binding proteins and chaperones. Structural proteins include actin and its binding proteins. Membrane proteins found include a G-beta protein, receptor fragments and the oligomycin sensitivity conferral protein of the mitochondrial ATP synthase. Imaging of labeled mite sections revealed that specific neuronal fibers stain most densely with the treatment and not with the controls. These neuronal fibers lead to either the front legs into sensory hairs or to the zone behind the fourth legs, to bristles on the edge of the mite's dorsal plate and to the air sac. Muscles stain lightly, perhaps due to actin binding. The mite's digestive or reproductive systems never stain with our probes, indicating that the compound interacts most strongly with specific components of the central and peripheral (sensory/motor) nervous system.