Oxalic Acid Derivatives for *Varroa* Control

Project No.: **07-POLL4-LeBlanc/Sammataro**
(LeBlanc no longer at this location)

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Objectives:

1) Synthesize OA derivatives (carboxylic ester and carboxylic amide)
2) Study the toxicity of the compounds (OA derivatives and BA derivatives) to *Varroa* mites using scintillation vial assays.

Interpretive Summary:

Honey bees are being parasitized by *Varroa destructor* worldwide, and the acaricides used for *Varroa* control (fluvalinate and coumaphos) have become ineffective. The mites' resistance to these chemicals is becoming widespread and beekeepers need another control alternative until the mite-resistant bee stock becomes more reliable. Oxalic acid (OA) has been used successfully in Europe to control *Varroa*. One method is to spray OA on the bees that are on broodless combs; mite mortality of 82-99% has been reported with this method (Imdorf et al. 1997; Milani 2001; Mossbeckhofer et al. 2003; Mutinelli et al. 1997). OA has been known to have varied levels of effectiveness against *Varroa* mites and undesired toxicity to the honey bee colony depending on the ambient conditions. Many beekeepers and scientists are now seeking alternatives to OA for this reason. For instance, in a low humidity and higher temperature environment, the amounts for the desired action would be less than a low temperature, high humidity area. This is because the vapor pressure of the chemicals is decreased. The work described here is an attempt to develop a compound with universal efficacy against *Varroa*, and make it more usable with fewer undesirable effects to honey bees. The first objective has been finished and we are just starting the second objective; it will be completed by the end of the year.

Materials and Methods:

1. Synthetic Chemistry Section for Synthesis of OA Derivatives

Since the carboxylic acid ester and amide derivatives are not commercially available we had to synthesize and characterize them ourselves. The oxalyl chloride (5 grams; 39.39

mmole) was mixed with 100 mL tetrahydrfuran (THF) in a 250 mL round bottom flask equipped with a magnetic stirring bar, an addition funnel and a calcium chloride drying tube. The contents were stirred in an ice bath (0^oC) and the corresponding alcohol or amine (39.39 mmol) was dissolved in 50 mL THF with exactly 39.39 mmol pyridine (dried over KOH). The alcohol or amine was dripped in at about 20 drops (1 mL) per minute and allowed to stir overnight. The next day the solvents were evaporated away at low pressure with a rotary evaporator apparatus and the residue dissolved in ethyl acetate. The ethyl acetate layer was extracted (3 x 30 mL) with a 2% HCl solution and then with water (1 x 30 mL) followed by 5% $NaHCO_3$ (3x 30 mL). The bicarbonate extracts were combined and acidified until the pH was 3; then they were extracted with ethyl acetate (3 x 30 mL). The ethyl acetate extracts were combined and dried over MgSO4, suction filtered, and the ethyl acetate evaporated to leave an oil residue which is crystallized with petroleum ether. The solid was characterized by melting point, infrared spectroscopy, nuclear magnetic resonance spectroscopy and by elemental analysis for % C, H and N.

Now that we have some compounds to test, we will proceed with Objective 2.

2. Mite Vial Assay

Varroa mites will be collected from frames containing capped brood (immature bees) taken from mite-infested honey bee colonies at our laboratory. The compounds of interest will be tested for mite-kill activity according to the vial protocol pioneered by Platt and Vinon (1977), modified for *Varroa* by Elzen and colleagues (1998). In brief, the brood cells are uncapped and the brood removed with forceps; then live adult female *Varroa* are collected with a slender probe. Mites are placed into 20-mL glass scintillation vials treated with either 0.5 mL acetone (Control), the compounds of interest (OA, OA derivatives, BA and derivatives); oxalic acid is the positive control.

Five mites will be placed into each vial. The mite vials will be incubated for 24 hours at approximately 30^oC and 80% relative humidity. A minimum of five replicate vials of each miticide/control will be tested for each colony and we will test each compound at 5 concentrations.

At 24 hours, we will examine the mite vials under a dissecting microscope. The mites will be gently prodded with a probe to encourage movement. Non-moving mites will be scored as dead and we will record mortality rates for each vial. For each colony tested, we will tally total mortality rates from all vials of each type: control (OA), the compounds of interest (OA derivatives, BA and BA derivatives).

From the mortality we will calculate a lethal dose (LD-50) for each compound. From these data, and the compounds that had high mite mortality, we will test them on honey be colonies infested with *Varroa*.

References:

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