Discovery of Resistance-Breaking Chemistries for Varroa Mite Management

Project No.: **14-POLL6A-Anderson**

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Objectives:

The objective of this study is to examine the acute toxicity and field efficacy of standard-use and stilbene acaricides to varroa mite populations.

Interpretive Summary:

The varroa mite is a primary driver for the periodical losses of honey bee colonies. The mite requires bees for food and reproduction and, in turn, elicits physiological deficiencies and disease transmission that compromise the health of bee colonies. The mite nervous system is a target site for existing acaricide chemistries. However, these acaricides not only have adverse health effects on bees, but resistance to these chemistries limits their use to reduce mite infestations and resulting disease transmission in bee colonies. Voltage-gated chloride channels are involved the maintenance of nerve and muscle excitability in arthropod pests, which suggests these channels might be exploited as target sites for unique acaricide chemistries. Apistan® (the pyrethroid tau-fluvalinate), Checkmite+™ (the organophosphate coumaphos), and Apivar® (the foramidine amitraz) are among several control products for management of varroa mites (Martel et al. 2007). The effectiveness of these chemistries has diminished as a result of the increasing incidence of either target-site or metabolic detoxification resistance in varroa mite populations, worldwide (González-Cabrera et al. 2013; Dmitryjuk et al. 2013). Here, we report a toxicological analysis of a natural stilbene product and related analogs against acaricide-susceptible and -resistant varroa mite populations.

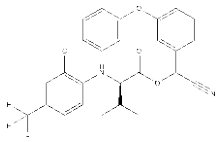
Materials and Methods:

- Honey bee colonies are maintained at the Virginia Tech. Nurse bees (6-10 d old adults) were collected for each toxicity and field efficacy bioassay. Standard chemical testing included technical grade *tau*-fluvalinate, coumaphos, and amitraz were purchased from ChemService Inc. (West Chester, PA). Commercial formulations of Apistan®, CheckMite+™, and Apivar® strips were purchased from Dadant & Sons Beekeeping Equipment. Test

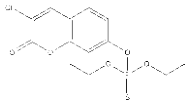
chemicals included 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), 2-methoxystilbene, 3,5-dimethoxystilbene, and (E)-2-(4-methoxystyryl)phenol that were purchased from Sigma-Aldrich Chemical Co.

- *tau*-Fluvalinate, coumaphos, amitraz, and stilbene toxicity was examined with honey bees and varroa mites using oral, topical, or dip exposure bioassays. Technical grade acaricides were orally administered in 50% sucrose solution or topically administered as a 1-µl aliquot to the dorsal thorax of each bee (ethanol carrier solvent). Each acaricide was tested at 10% active ingredient on 10 bees per treatment. Percent mortality was assessed 24 h post-treatment. Less than 10% mortality was observed for the honey bees exposed to each oral and topical treatment of acaricide. Each acaricide was tested at six concentrations on ten varroa mites per treatment. Each honey bee and varroa mite treatment was replicated three times.
- Field efficacy of *tau*-fluvalinate, coumaphos, amitraz, and stilbenes was assessed with acaricide-susceptible and -resistant varroa mite populations using contact exposure bioassays. Bioassay arenas consisted of a 750-ml plastic container fastened to a wood platform with a removable sticky board. For each bioassay, *ca*. 300 honey bees were collected from the brood frame(s) of a hive. Bioassay arenas contained a 3 x 3 cm tab impregnated with *tau*-fluvalinate (Apistan®, 10.2% ai), coumaphos (CheckMite+™, 10.0% ai), or amitraz (Apivar®, 3.3% ai) or treated with the stilbenes (10% ai). Bioassay arenas were transported to the laboratory and maintained in a dark environmental chamber at 32 °C to measure the time-dependent efficacy of each acaricide. Number of varroa mites on the sticky boards was counted after a 3- and 6-h acaricide exposure period and the remaining varroa mites attached to the honey bees were counted after a 3 min. ethanol wash.

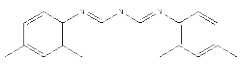
Results and Discussion:



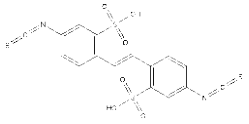
tau-Fluvalinate



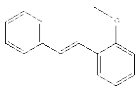
Coumaphos



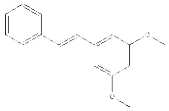
Amitraz



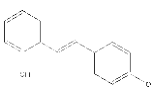
DIDS



2-Methoxystilbene



3,5-Dimethoxystilbene



(E)-2-(4-Methoxystyryl)Phenol

Table 1. Acute toxicity of standard-use and stilbene acaricides to varroa mites.

Acaricide	LC_{50} (µM) 95% CI	SLOPE ± SE	X^2
tau-Fluvalinate	8.51 2.87 - 27.75	0.73 ± 0.13	8.57
Coumaphos	2.22 0.55 - 9.25	0.48 ± 0.07	15.60
Coumaphos-oxon	0.66 0.06 - 5.45	0.58 ± 0.13	13.77
Amitraz	0.70 0.23 - 2.34	0.80 ± 0.14	15.47
DIDS	154.20 16.75 - 2271.78	0.29 ± 0.06	14.73
2-Methoxystilbene	2097.34 231.41 - 180336.50	0.31 ± 0.07	10.25
3,5-Dimethoxystilbene	141.31 35.25 - 887.90	0.47 ± 0.08	12.20
(E)-2-(4-Methoxystyryl)Phenol	6424.73 479.67 - 2824129.20	0.29 ± 0.07	11.97

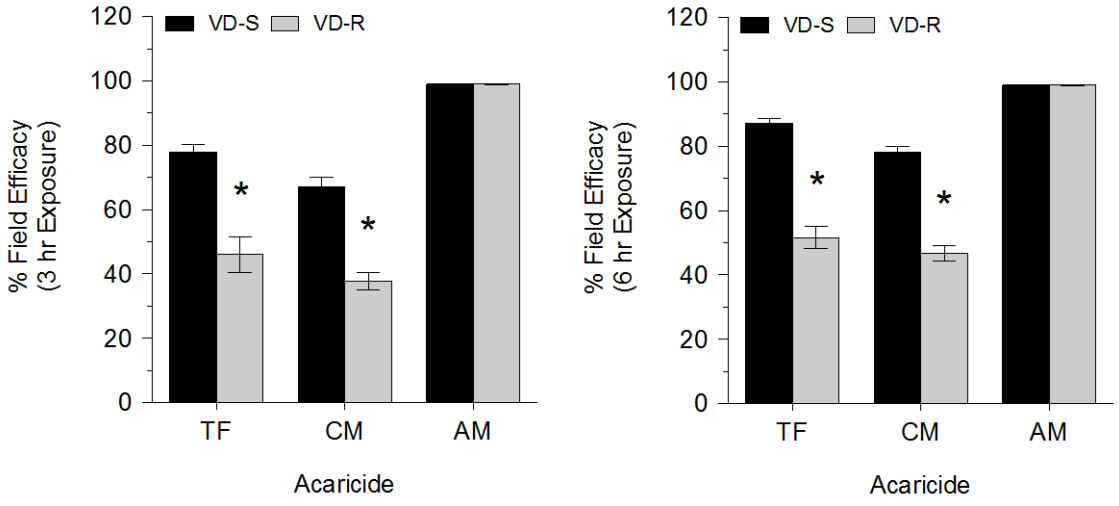


Figure 1. Field efficacy bioassays of *tau*-fluvalinate (TF, Apistan®), coumaphos (CM, CheckMite+™), and amitraz (AM, Apivar®) against acaricide-susceptible and –resistant varroa mite populations. Acaricide efficacy was assessed for the varroa mite populations after a 3- and 6-h exposure period. Varroa mites remaining on the honey bees were removed after a 3-min ethanol wash. Acaricide efficacy < 60% suggests the possibility of resistance after a 6-h exposure period. Each test was considered valid based on the presence of > 5 varroa mites per 100 honey bees. * $P < 0.05$, $n = 10$

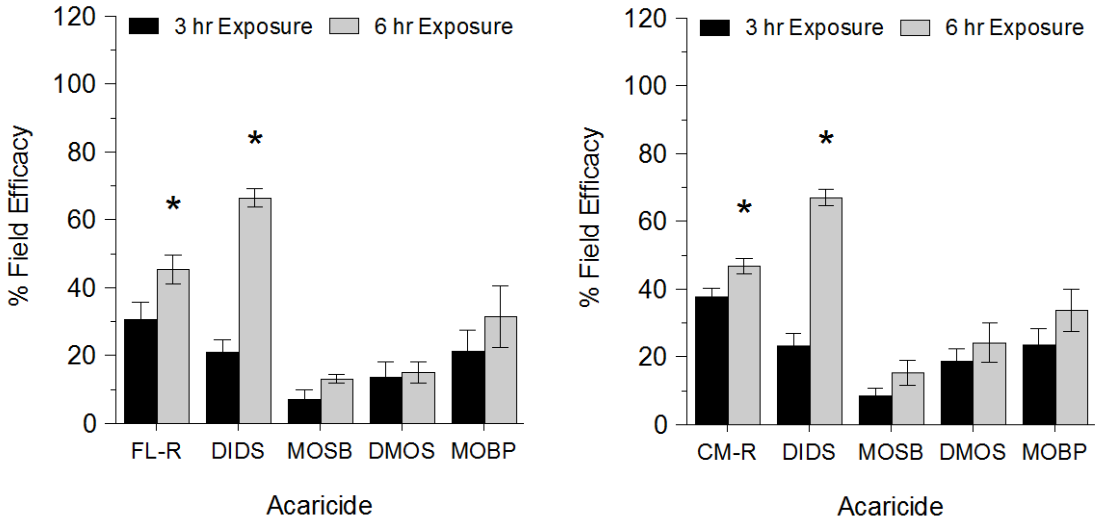


Figure 2. Field efficacy bioassays DIDS, 2-methoxystilbene, 3,5-dimethoxystilbene, and (E)-2-(4-methoxystyryl)phenol) against coumaphos-resistant varroa mite populations. Stilbene (10.0% ai) efficacy was assessed for *tau*-fluvalinate (FL-R) coumaphos-resistant (CM-R) varroa mite populations after a 3- and 6-h exposure period. Varroa mites remaining on the honey bees were removed after a 3-min ethanol wash. Stilbene efficacy < 60% suggests the possibility of tolerance after a 6-h exposure period. Each test was considered valid based on the presence of > 5 varroa mites per 100 honey bees. Varroa mite populations were selected based on their resistance to coumaphos (**Figure 1**). * $P < 0.05$, $n = 6$

- Apistan® (the *tau*-fluvalinate pyrethroid), Checkmite+™ (the organophosphate coumaphos), and Apivar® (the foramidine amitraz) were used as standard-use acaricides. Both *tau*-fluvalinate and coumaphos have lower field efficacy than amitraz to the tested varroa mite populations. This suggests that the varroa mites are resistant to *tau*-fluvalinate and coumaphos based on the percent efficacies being less than 60% (**Figure 1**).
- Acute toxicity of the standard-use acaricides *tau*-fluvalinate, coumaphos, and amitraz is greater than that of test chemical DIDS to acaricide-susceptible varroa mites (**Table 1**).
- *tau*-Fluvalinate and coumaphos-resistant varroa mite populations appear to be tolerant to the test stilbenes 2-methoxystilbene, 3,5-dimethoxystilbene, and (E)-2-(4-methoxystyrl)phenol with field-efficacy percentages less than 60% (**Figure 2**). However, the test stilbene DIDS exhibited field efficacy higher than 60% against both acaricide-resistant varroa mite populations compared to *tau*-fluvalinate and coumaphos (**Figure 2**). These data suggest that DIDS might serve as candidate chemistry for the development of alternative acaricides for *tau*-fluvalinate- and coumaphos-resistant varroa mite populations.
- Current research activities are focused on the acaricide-resistance monitoring and management, identification of metabolic detoxification and target-site resistance mechanisms, and discovery of unique chemistries with acaricidal activity for the management of varroa mite populations and the protection of honey bee health.

Research Effort Recent Publications:

N/A

References Cited:

Dmitryjuk, M., Zoltawska, K., Fraczek, R., and Lipinski, Z. 2014. Esterases of *Varroa destructor* (Acari: Varroidae), parasitic mite of the honeybee. Exp. Appl. Acarol. 62(4):499-510.

González-Cabrera, J., Emyr Davies, T. G., Field, L. M., Kennedy, P. J., and Williamson, M. S. 2013. An amino acid substitution (L925V) associated with resistance to pyrethroids in *Varroa destructor*. DOI: 10.1371/journal.pone.0082941.

Martel, A. C., Zeggane, S., Aurieres, C., Drajnudel, P., Faucon, J.P., Aubert, M. 2007. Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar (R) or Asuntol (R) 50, Apidologie 38:534–544.