Discovery of Resistance-Breaking Acaricides for Varroa Mite Management

Project No.: **15-POLL17-Anderson**

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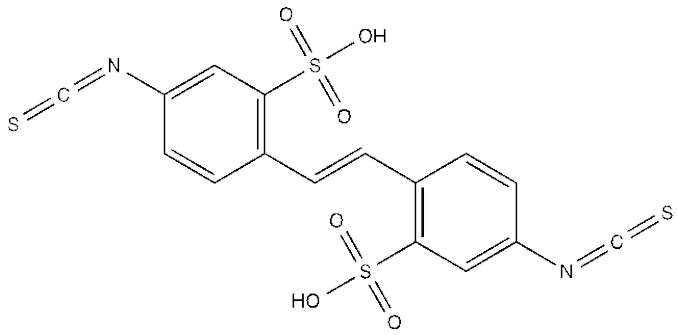
Objectives:

1. Survey the field efficacy of acaricide products to varroa mite populations
2. Characterize the metabolic detoxification mechanisms of acaricide resistance in varroa mite populations.
3. Examine the field efficacy of stilbene acaricides to resistant varroa mite populations

Interpretive Summary:

The hematophagous mite, *Varroa destructor*, is a major pest of the honey bee, *Apis mellifera*. It is considered to be a primary driver for the periodical losses of managed bee colonies in the US. The varroa mite is an ectoparasite that depends on its host, the honey bee, for food and reproduction and, in turn, elicits physiological deficiencies and vectors viruses that can decrease the number of healthy bee colonies available for pollination services. The most widespread and effective approach to mite management is through the use of acaricide products, such as Apistan® (the pyrethroid *tau*-fluvalinate), CheckMite+™ (the organophosphate coumaphos), or Apivar® (the formamidine amitraz) alone or in combination with other softer compounds including organic acids and essential oils. These products target the central nervous system of mites, but have adverse health effects on adult and immature worker bees, particularly as acaricide residues persist and accumulate over time in the brood



DIDS

combs and other hive resources. In addition, the development of mite resistance to these acaricides limits their use to reduce the risk of mite infestations and vectored viruses in bee colonies.

Voltage-gated chloride channels (VGCCs) are important for the maintenance of electrical excitability in nerve and muscle membranes of arthropods and, thus, serve as an exploitable target site for alternative acaricide chemistries. The natural product stilbenes, isolated from the bacterium *Photorhabdus luminescens*, elicit paralytic activity by blocking VGCCs in arthropod pests. These stilbene chemistries provide a unique opportunity for the discovery and development of alternative acaricides for resistant mite populations. Here, we report that the stilbene DIDS provides an alternative acaricide intervention to incapacitate or deplete acaricide-resistant mite populations in bee colonies.

Materials and Methods:

Objective 1 - Survey the field efficacy of acaricide products to varroa mite populations**.** To determine the field efficacy of the acaricide products to the mite populations, a bioassay arena consisting of a 750 ml plastic container fastened to a wood platform with a removable sticky board was used to collect ~ 300 bees from the brood frame(s) of a bee hive. Apistan® (10.2% *tau*-fluvalinate active ingredient (ai)), CheckMite+™ (10.0% coumaphos ai), or Apivar® (3.3% amitraz ai) was assessed with mite populations using contact exposure bioassays. The bioassay arenas contained a 3.0 x 3.0 cm tab impregnated with Apistan®, CheckMite+™, or Apivar®. The bioassay arenas were placed in the dark of a ventilated styrofoam box and the effective endpoint was measured as the number of mites collected on the sticky board per 100 bees in the bioassay arena following a 3- and 6-h exposure period. The bees were washed with ethanol and agitated for 3 min following the 3- and 6-h exposure period to remove any additional mites left on the bees. The remaining mites were quantified to evaluate the percent efficacy of the acaricide product(s) (i.e., calculated as the number of mites collected on the sticky board during acaricide treatment divided by the number of mites remaining on the bees following the 3- and 6-h exposure period). The level of acaricide tolerance or resistance was documented if the chemistry has ≤ 60% field efficacy to a mite population. In other words, the susceptibility/resistance status of tested mite populations is defined as confirmed susceptibility to acaricides at 100% - 60% mortality and confirmed resistance at 59% - 0% mortality.

Objective 2 - Characterize the metabolic detoxification mechanisms of acaricide resistance in varroa mite populations. To evaluate detoxification capacity in acaricide-susceptible and -resistant mites, the metabolic activities of cytochrome P450 monooxygenase (P450), esterase (Est), and glutathione *S*-transferase (Gst) enzymes were assessed using adult mites collected from sticky boards during the field efficacy bioassays (Objective 1). These data suggest that the elevated Est, Gst, and P450 enzyme activities in the resistant mites may increase the metabolic detoxification of the acaricides Apistan® and CheckMite+™ and, thereby, reducing their efficacy to mite populations.

Objective 3 - Examine the field efficacy of stilbene acaricides to resistant varroa mite populations**.** To determine the field efficacy of the stilbenes to the acaricide-resistant mite populations (Objectives 1 and 2), a bioassay arena consisting of a 750 ml plastic container fastened to a wood platform with a removable sticky board was used to collect ~ 300 bees

from the brood frame(s) of a bee hive. The stilbene DIDS, and related analogs, (10% a.i.) were assessed with mite populations using contact exposure bioassays. The bioassay arenas contained a 3.0 x 3.0 cm tab treated with the stilbenes. The bioassay arenas were placed in the dark of a ventilated styrofoam box and the effective endpoint was measured as the number of mites collected on the sticky board per 100 bees in the bioassay arena following a 3- and 6-h exposure period. The bees were washed with ethanol and agitated for 3 min following the 3- and 6-h exposure period to remove any additional mites left on the bees. The remaining mites were quantified to evaluate the percent efficacy of the stilbenes (i.e., calculated as the number of mites collected on the sticky board during stilbene treatment divided by the number of mites remaining on the bees following the 3- and 6-h exposure period). The level of stilbene effectiveness was documented if the chemistry has $\geq$ 60% field efficacy to an acaricide-resistant mite population.

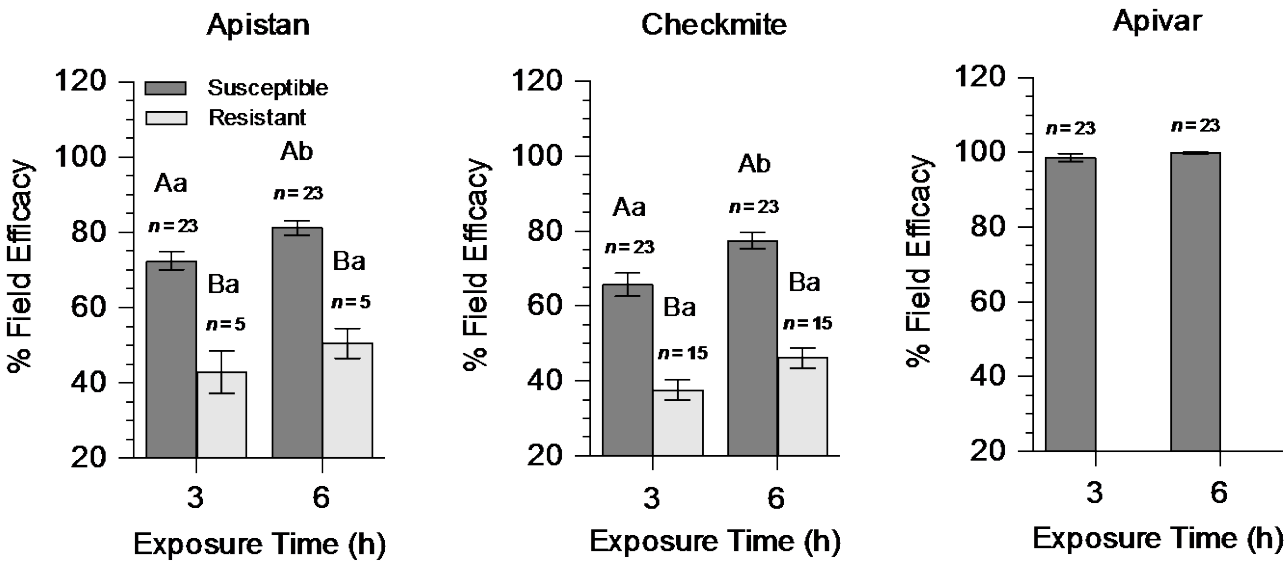


Figure 1. Field efficacy of Apistan® (*tau*-fluvalinate, 10.2% ai), CheckMite+® (Coumaphos, 10.0% ai), and Apivar® (Amitraz, 3.3% ai) against varroa mite populations. Vertical bars indicate standard errors of the mean. Acaricide efficacy < 60% suggests the possibility of resistance after a 6-h exposure period. Different letters on the bars indicate that the means are significantly different among the susceptible and resistant mites (uppercase) or acaricide exposure times (lowercase) using a one-way ANOVA and LSD multiple comparison test. Mite populations were sampled in Blacksburg, VA. No Apivar resistant mites were detected in the samples. *n* = number of acaricide susceptible or resistant mite populations

Results and Discussion:

Acaricide resistance is a serious pest management challenge that warrants the development of improved acaricide interventions for mite populations in bee colonies. The acaricides Apistan®- and CheckMite+™ have significantly lower field efficacy than Apivar® to the tested mite populations in bee colonies (**Figure 1**). These data suggest that the mites are resistant to the active ingredients *tau*-fluvalinate and coumaphos based on the percent efficacies being less than 60% (**Figure 1**). The characterization of metabolic detoxification mechanisms in mite populations allows for the confirmation of acaricide resistance and the development of intervention strategies to manage acaricide resistance. The significantly higher Est, Gst, and P450 enzyme activities in the acaricide-resistant mites compared to those of susceptible mites suggests a metabolic detoxification mechanism(s) of resistance to Apistan® and CheckMite+™

and, thereby, reduces their field efficacy to the tested mite populations in bee colonies (**Figure 2**). The stilbene DIDS has significantly higher field efficacy to the acaricide-resistant mite populations in bee colonies compared to that of Apistan® and CheckMite+™ (**Figure 3**). The stilbene DIDS provides an alternative acaricide intervention to incapacitate or deplete acaricide-resistant mite populations and, in turn, warrants the continued development of these stilbene-based chemistries for use in bee colonies.

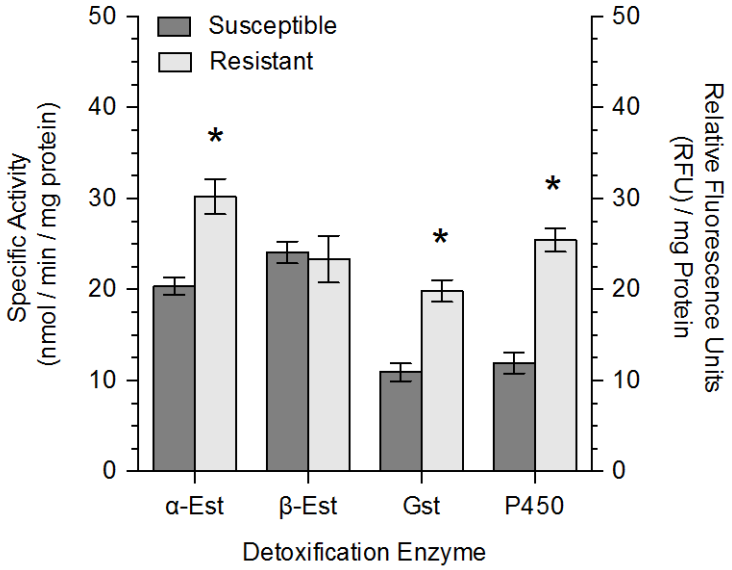


Figure 2. Comparison of general esterase (Est), Glutathione-*S*-transferase (Gst), and cytochrome P450-dependent *O*-deethylation (P450) activities of acaricide-susceptible and -resistant varroa mites. Vertical bars indicate standard errors of the mean ($n = 10$), asterisks (*) denote means that are significantly different between the acaricide-susceptible and -resistant mites (students *t*-test, $P < 0.05$). α-naphthyl acetate (α-NA), β-naphthyl acetate (β-NA), 1-chloro-2, 4-dinitrobenzene (CDNB), 7-ethoxycoumarin (7-EC).

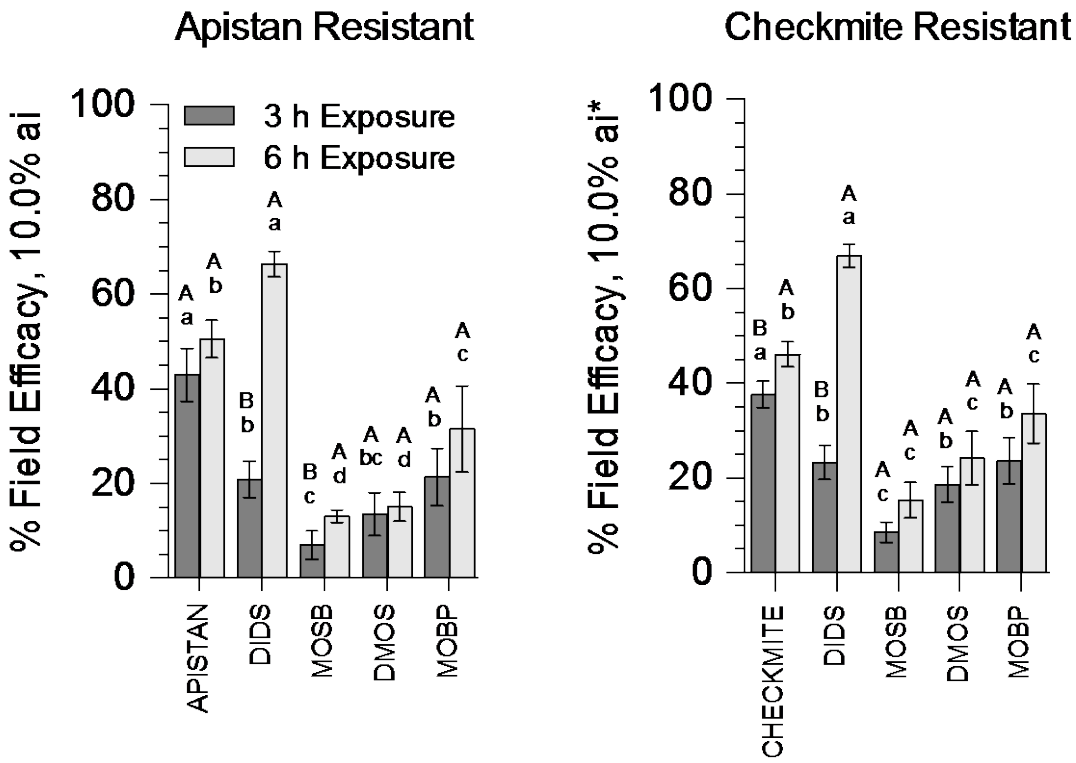


Figure 3. Field efficacy of the stilbene DIDS and related analogs (MOSB, DMOS, MOBP) against acaricide-resistant mite populations. Vertical bars indicate standard errors of the mean. Different letters on the bars indicate that the means are significantly different among the exposure periods (uppercase) or acaricide treatments (lowercase) using a one-way ANOVA and LSD multiple comparison test. *n* = 5 Apistan-resistant mite populations tested; *n* = 15 CheckMite-resistant mite populations tested

Research Effort Recent Publications:

Vu, P. D., Jenson, L. J., Bloomquist, J. R., and Anderson, T. D. 2016. Stibenes actives for varroa mite management. *Arthropod Management Tests* (In review).