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ROLE OF DETOXIFICATION IN VARROA DESTRUCTOR (ACARI: VARROIDAE) TOLERANCE OF THE MITICIDE TAU-FLUVALINATE

Reed M. Johnson1,†, Zachary Y. Huang2 and May R. Berenbaum1
1. Department of Entomology, University of Illinois at Urbana-Champaign, 320 Morrill Hall, 505 S. Goodwin Avenue, Urbana, IL 61801, USA (e-mails: rmjohns1@gmail.com and maybe@illinois.edu); 2. Department of Entomology, Michigan State University, 243 Natural Science, East Lansing, MI 48824, USA (e-mail: bees@msu.edu).

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ABSTRACT – The varroa mite (Varroa destructor Anderson and Trueman) is a devastating pest of honey bees (Apis mellifera L.). Beekeepers have relied on the pyrethroid pesticide tau-fluvalinate as a principal agent of varroa mite control. While this miticide was quite effective at controlling varroa mites through the 1990s, its efficacy has waned as resistance to tau-fluvalinate has appeared in many populations of mites. Resistance in some populations of varroa mites has been associated with elevated detoxification of tau-fluvalinate. Honey bees tolerate miticidal tau-fluvalinate applications principally through rapid detoxification mediated by cytochrome-P450 mono-oxygenases, with the other detoxification enzyme families, the carboxylesterases and glutathione-S-transferases, playing much smaller roles in miticide tolerance. The goal of this study was to test the capability of the glutathione-S-transferase enzyme inhibitors diethyl maleate and curcumin, which should interfere minimally with honey bee detoxification, to elevate the toxicity of tau-fluvalinate to a population of varroa mites. Additionally, to test the role of cytochrome P450s and esterases in any detoxification-mediated resistance, varroa mites were also treated with the enzyme inhibitors piperonyl butoxide and S,S,S-tributyl phosphorotrithioate. None of the tested enzyme inhibitors increased the toxicity of tau-fluvalinate, suggesting that detoxification plays a minimal role in the tolerance of tau-fluvalinate in the population of varroa mites in this study.

Key words – Glutathione-S-transferase, varroa, Apis mellifera, pesticide resistance, pyrethroid, cytochrome P450 mono-oxygenase, curcumin.

INTRODUCTION

The Western honey bee (Apis mellifera L.), is among the most economically important insect species in North America; beyond its contributions to the US economy in the form of goods such as honey, wax, propolis, and pollen, the value of its service as a pollinator of plants used as food for humans and livestock exceeds $15 billion annually (Morse and Calderone, 2000). In recent years, the economic contribution of honey bee colonies has been threatened by colony collapse disorder (CCD) (Oldroyd, 2007). Although the cause of CCD has yet to be determined, the general consensus is that bee deaths from CCD are likely to be the result of a combination of diseases, parasites, and exposure to environmental toxicants (Watanabe, 2008). One ectoparasite of honey bees, the varroa mite (Varroa destructor Anderson and Trueman), has been killing colonies in the US since its introduction in the 1980s (Watanabe, 1994). As mite populations build up in a colony over several years, the bees, if untreated, will ultimately succumb to “parasitic mite syndrome” and the entire colony may die. Individual bees are directly harmed by varroa inasmuch as the mites suck...
hemolymph and parasitized bees are at increased risk of infection from the viruses vectored by mites and from the immnosuppression caused by varroa parasitism (Yang and Cox-Foster, 2005; Boecking and Genersch, 2008). Varroa mites may well be a contributing factor to CCD through their ability to transmit bee viruses (Yang and Cox-Foster, 2005), including Israeli acute paralysis virus, which may be associated with CCD (Chen and Evans, 2007; Cox-Foster et al., 2007).

Beekeepers often use in-hive miticides to treat infested colonies and prophylactically to keep varroa populations low. In 1992, the first synthetic miticide, Apistan®, was registered for use in the USA. Apistan® treatment consists of suspending plastic strips impregnated with the pyrethroid pesticide tau-fluvalinate between frames within the hive for 6–8 weeks, during which time the pesticide is slowly released onto the bees that contact the strip. Tau-fluvalinate is a subset of isomers of fluvalinate; as a pyrethroid it inhibits deactivation of voltage-gated sodium channels (Davies et al., 2007) and can kill mites at low concentrations and bees at substantially higher concentrations (Atkins, 1992).

Key to the success of tau-fluvalinate as an in-hive miticide is its relatively low toxicity to honey bees (Santiago et al., 2000). As a class, the pyrethrins include some of the most toxic of all pesticides to honey bees (Atkins, 1992). Cytochrome P450 mono-oxygenases (P450s) and carboxylesterases (COEs) play a role in pyrethroid metabolism in bees (Pilling et al., 1995). A bioassay study using inhibitors of P450s, COEs, and glutathione-S-transferases (GSTs) with tau-fluvalinate and other pyrethroids demonstrated that P450s are of primary importance in detoxifying these pesticides, with COEs and GSTs playing a lesser role (Johnson et al., 2006).

Although tau-fluvalinate was initially quite effective at controlling mite infestations and exhibited low toxicity to bees, ostensibly because of rapid P450-mediated detoxification (Atkins, 1992; Johnson et al., 2006), varroa mite populations rapidly developed resistance wherever tau-fluvalinate was used (Lodesani et al., 1995; Milani, 1995; Elzen et al., 1998; Mozes-Koch et al., 2000; Goodwin et al., 2005). Acquisition of resistance by mites was probably aided by the long-term low-dose exposure associated with Apistan® treatment, although resistance has also appeared in varroa populations with no history of miticide exposure (Goodwin et al., 2005; Sammataro et al., 2005).

The mechanism of tau-fluvalinate resistance in varroa doubtless follows the mechanisms of pyrethroid resistance that have developed in other pest arthropods: altered behavioral response, decreased penetration through the cuticle, decreased target site sensitivity, and elevated detoxification (Scott and Georgiou, 1986; Casida and Quistad, 1998). In varroa, mutations in the sodium channel target site have been identified that may contribute to resistance (Wang et al., 2002). In terms of the contribution of detoxification enzymes to resistance, the fact that mites possess cytochrome P450 mono-oxygenases, GSTs, and COEs has been confirmed, but the role of these enzymes in resistance is not clear.

Esterase activity has been confirmed in mites (Gerson et al., 1991). Whereas no difference in enzyme activity was observed between resistant and susceptible strains in studies using the model substrate naphthyl acetate (Wu et al., 2003; Sammataro et al., 2005), a 2.5-fold increase in esterase activity was documented in resistant mites using p-nitrophenyl acetate as a substrate (Mozes-Koch et al., 2000). No P450 activity was observed in varroa when methoxyresorufin (Wu et al., 2003), benzo(α)pyrene, or benzphetamine was used as a model substrate (Mozes-Koch et al., 2000); p-nitrophenol, however, was metabolized and assays using resistant mites demonstrated a 20-fold increase in p-nitroanisole activity (Mozes-Koch et al., 2000). Aryl hydrocarbon hydroxylase activity, based on another model P450 substrate, has also been reported in mites (Baars and Driessen, 1984). Bioassays using piperonyl butoxide (PBO) as an inhibitor of P450-mediated detoxification of tau-fluvalinate have produced similarly mixed results, with one study demonstrating a substantial increase in the toxicity of tau-fluvalinate in resistant populations of mites when treated with PBO, but only a minor increase in toxicity in susceptible populations (Hillesheim et al., 1996). Other studies found no synergism between PBO and tau-fluvalinate in either susceptible or resistant mites (Bell et al., 1999; Wu et al., 2003). Early work demonstrated GST activity in varroa through the conjugation of glutathione to 1-chloro-2,4-dinitrobenzene (CDNB), a model substrate (Baars and Driessen, 1984; Gerson et al., 1991). No difference in conjugation of CDNB was observed between resistant and susceptible strains of mites (Wu et al., 2003). Bioassays using N-ethylmaleimide and diethylmaleate (DEM) found no synergistic increase in the toxicity of tau-fluvalinate to resistant mites (Bell et al., 1999). However, DEM treatment increased the toxicity of pyrethroids to other mite species, suggesting that GST-mediated detoxification plays a central role in pyrethroid tolerance in mites (Yang et al., 2001).

As both honey bees and mites rely on a diversity of biochemical and physiological mechanisms for detoxification of pesticides, identifying taxon-specific resistance mechanisms can provide important tools for management of resistance and continued use of chemically based management approaches. To clarify
and applied to vials. Solvent was allowed to evaporate as the vials were constantly rolled using a hot dog roller (HDG-598, The Helman Group, Oxnard, CA). Tau-fluvalinate (Chem Services) in acetone (12–6000 ng/vial) was then added to the vials and the solvent was again allowed to dry.

Twenty mites were placed inside each prepared vial and provided with a worker pupa for food 6 h later. Mortality was scored after 24 h, with mites that showed no response when contacted with a metal probe scored as dead. A log-probit line was fitted for each treatment from which the LC$_{50}$ values and 95% confidence intervals were calculated using the $R$ statistical package (R Development Core Team, 2008) with correction for heterogeneity when needed (Finney, 1971). Treatments with non-overlapping 95% confidence intervals are considered to demonstrate significant differences in toxicity.

**RESULTS AND DISCUSSION**

**Inhibitor toxicity** – Curcumin did not cause mite mortality at any tested concentration, even at the highest concentration, 1000 μg/vial. Diethyl maleate displayed intermittent toxicity at very high concentrations, with a calculated LC$_{50}$ of 300.3 μg/vial; however, this quantity of liquid synergist probably killed some mites by physical submersion. Calculated LC$_{50}$ values for DEF and PBO were 33.3 μg/vial and 73.5 μg/vial, respectively.

**Measuring resistance** – The population of mites evaluated in this study appears to be mildly resistant to tau-fluvalinate, with an LC$_{50}$ of 384 ng/vial. Using the same bioassay technique, Wu et al. (2003) established an LC$_{50}$ of 127 ng/vial for susceptible mites from Nebraska and 777–2957 ng/vial for mites from a resistant population from Cable, Wisconsin. Elzen et al. (1998) estimated the LC$_{90}$ for susceptible mites from Texas using this assay at 2.4 μg/vial. The LC$_{90}$ for the population of mites used in this study is 8.99 μg/vial (3.71–41.25 μg/vial), suggesting that this population of varroa mites is somewhat resistant to tau-fluvalinate. It is difficult to compare tau-fluvalinate toxicity statistics with others reported, as much previous work was performed using a different method in which tau-fluvalinate was incorporated into paraffin wax (Milani, 1995). The relatively shallow slope of the fitted probit line, 0.91 ± 0.06, suggests heterogeneity in the resistance of individual mites. Hillesheim et al. (1996) found the slope of the probit line was much steeper, 4.5 ± 0.8 and 6.7 ± 1.1, for two susceptible strains of mites, but similarly shallow, 1.6 ± 0.3 and 2.8 ± 0.5, for resistant strains. Clearly, resistance to tau-fluvalinate is widespread, relative to the susceptibility exhibited by mites historically, and
appears to be present to some degree in the apiary used in this study despite the fact that Apistan® treatment had not been used for several years.

**Synergism** – No significant differences were observed in the toxicity of tau-fluvalinate to mites exposed to any of the inhibitor treatments (Table 1). This result indicates that detoxification contributes little to the tolerance of tau-fluvalinate in this population. Lack of synergism between PBO and tau-fluvalinate supports the findings of some previous bioassays using both susceptible and resistant mites (Bell et al., 1999; Wu et al., 2003), but it conflicts with the substantial synergism reported by others (Hillesheim et al., 1996; Mozes-Koch et al., 2000). This may be evidence that different resistant populations of varroa mites carry different mechanisms of resistance. Alternatively, differences in PBO synergism could be the result of differences in methods of PBO administration. Hillesheim et al. (1996) found synergism when PBO was applied topically to the dorsum of mites, but studies in which PBO has been administered indirectly to mites on the surface of a vial, including the present study, have failed to find PBO-synergism. To be practical, any in-hive synergist must be applied at the same time as the tau-fluvalinate treatment. It is possible that P450s could play a role in tau-fluvalinate resistance, but high concentrations of PBO or other P450 inhibitors would be required to restore the toxicity of tau-fluvalinate. Such concentrations, based on the results of past studies, would also inhibit honey bee metabolism of tau-fluvalinate and greatly increase the toxicity of this miticide to bees (Johnson et al., 2006).

Although COE enzyme activity has been found in varroa mites, at best only a weak synergism has been found between DEF and tau-fluvalinate in bioassays (Hillesheim et al., 1996). Lack of synergism could also be the result, as with PBO, of differences in inhibitor administration, but it seems unlikely that COEs actually make any substantial contribution to tau-fluvalinate resistance.

Both DEM and curcumin failed to synergize tau-fluvalinate at the concentrations tested. Both compounds appear to be relatively non-toxic to mites in the absence of tau-fluvalinate, suggesting that GSTs play a minor role in both the detoxification of tau-fluvalinate and mite survival in general. Curcumin, as the only solid inhibitor, was probably poorly absorbed through the cuticle of mites. It even appeared to slightly decrease the toxicity of tau-fluvalinate, probably because as a powder it formed a solid crust protecting mites from the tau-fluvalinate on the vial.

Inhibition of detoxifying enzymes failed to increase the toxicity of tau-fluvalinate to this population of varroa mites. This finding suggests that insensitivity of the voltage-gated sodium channel to tau-fluvalinate (Wang et al., 2002; Liu et al., 2006) is the basis for the elevated tolerance of tau-fluvalinate. That other populations of mites appeared to achieve resistance through detoxification indicates that varroa mites may be capable of multiple forms of resistance to tau-fluvalinate.

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