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Acute Contact Toxicity of Oxalic Acid to Varroa destructor (Acari: Varroidae) and Their Apis mellifera (Hymenoptera: Apidae) Hosts in Laboratory Bioassays

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ABSTRACT Laboratory bioassays were performed to characterize the acute contact toxicity of oxalic acid (OA) to Varroa destructor (Anderson & Trueman) and their honey bee hosts (Apis mellifera L.). Specifically, glass-vial residual bioassays were conducted to determine the lethal concentration of OA for V. destructor, and topical applications of OA in acetone were conducted to determine the lethal dose for honey bees. The results indicate that OA has a low acute toxicity to honey bees and a high acute toxicity to mites. The toxicity data will help guide scientists in delivering optimum dosages of OA to the parasite and its host, and will be useful in making treatment recommendations. The data will also facilitate future comparisons of toxicity if mite resistance to OA becomes evident.

KEY WORDS Varroa destructor, Apis mellifera, oxalic acid, bioassay, LD₅₀

Varroa destructor (Anderson & Trueman) (Acari: Varroidae), an obligate ectoparasitic mite of the honey bee, Apis mellifera L. (Hymenoptera: Apidae), feeds on the hemolymph of adult bees and of larvae and pupae in capped brood cells. Severe V. destructor infestation of A. mellifera results in honey bee colony death within 3 to 4 yr of initial infestation (Ritter et al. 1983). In 1987, V. destructor was detected in the United States (Anonymous 1987) and has subsequently spread to most regions of the country. At present, V. destructor is the most serious pest of managed honey bee colonies worldwide (Anderson and Trueman 2000).

Miticides are frequently used to control V. destructor in honey bee colonies, and most beekeepers routinely treat their colonies to reduce mite populations. Mite resistance to fluvalinate (Apistan) and coumaphos (Checkmite+), the two most widely applied chemical treatments, has become a major problem for beekeepers in the United States and Europe. Resistance to the pyrethroid fluvalinate has been documented in the United States (Eischen 1995, 1998; Elzen et al. 1998, 1999; Macedo et al. 2002) and Europe (Milani 1994, Lodesani et al. 1995, Thompson et al. 2002). Similarly, resistance to the organophosphate coumaphos has been reported in the United States (Elzen and Westervelt 2002) and Italy (Lodesani 1996). The lipophilic nature of fluvalinate and coumaphos enables them to accumulate in beeswax combs (Wallner 1999). The risk of hive contamination and the decreased efficacies of current V. destructor treatments create a need for alternative control strategies.

Oxalic acid (OA) is widely used for controlling V. destructor in Europe and Canada, because of its high efficacy (>90%) and low risk of hive contamination (Charrière and Imdorf 2002, Special Supplement 2005). OA is applied to colonies by spraying or trickling a solution of OA and sugar water over the bees (Charrière and Imdorf 2002) or by evaporating crystals with heat. When trickling OA in Canada, the recommended application per hive is 50 ml of a solution containing 35 g of OA dihydrate in 1 liter of 1:1 sugar:water (wt:vol). When evaporating OA in Canada (vaporizer method), the recommended application is 2 g of OA dihydrate per hive (Special Supplement 2005). Although OA provides effective control of V. destructor, its mode of action is unknown. Furthermore, only one study has quantified the contact toxicity of OA to V. destructor (Milani 2001), and the contact toxicity of OA to honey bees has not been determined. In short, OA is extensively used without knowing the basic toxicological properties of the compound to V. destructor or A. mellifera.

The objective of this study was to characterize the acute contact toxicity of OA to V. destructor and their honey bee hosts in laboratory bioassays. The results will establish dosage–mortality relationships for populations of both mites and bees that have not been exposed to OA and will be useful in making treatment recommendations. The data also will facilitate future comparisons of toxicity if mite resistance to OA becomes evident.

Materials and Methods

Collection of V. destructor. Adult worker bees were collected from a single, mite-infested colony of Carniolan honey bees located on the University of Ne-
braska campus in February 2005. The bees from each frame (12 frames total) were shaken through a funnel into a bulk bee box. The bees were then subdivided into six smaller wooden boxes that measured 17.75 cm in length by 15.25 cm in width by 10.15 cm in depth (i.d.). Each box had an 8-screen on one of the 17.75- by 15.25-cm sides for ventilation and mite collection. A pint jar of sugar water (1:1 by volume) was attached to each box, and the boxes were stored at 15.6°C in complete darkness until needed for experimentation (no > 2 d).

Mites were harvested from adult bees by applying 18 g of powdered sugar through the screen of each box (Aliano and Ellis 2005). The boxes were inverted after bees were thoroughly coated with powdered sugar to collect mites (~30 s). The boxes remained inverted until mite fall ceased (~20 min). Approximately 100 viable mites were recovered per cage. The powdered sugar was gently brushed off mites with a fine paint brush before transferring them to 20-ml glass scintillation vials (Wheaton Scientific, Millville, NJ) for OA exposure.

Glass-Vial Residual Bioassays for *V*. *destructor*. Techniques described by Plapp and Vinson (1977) and Macedo et al. (2002) were used for conducting glass-vial residual bioassays. Serial dilutions of OA dihydrate (>99% purity) (The Science Company, Denver, CO) (CAS no. 6153-56-6) in acetone were prepared, and a preliminary range-finding bioassay was conducted to determine at least three concentrations that provided *V*. *destructor* mortalities >0 and <100%. Seven concentrations of OA in acetone were prepared for the definitive bioassay (1.0, 0.3, 0.1, 0.03, 0.01, 0.003, and 0.001 mg/ml). One-half milliliter of each solution was pipetted into four 20-ml glass scintillation vials for each treatment, including an acetone control (acetone only) was included in the bioassay as a replication and four vials (40 mites) were tested for each treatment. Furthermore, it was impossible to calculate the 24- and 48-h LD50 values for honey bee bioassay data were expressed as micrograms per vial. The doses used for Probit analysis of mite bioassay data were used for Probit analysis of the deﬁnitive bioassay data, because signiﬁcant mortality did not occur until at least 72 h posttreatment. Aliquots of a 200 mg/ml solution of OA in acetone were used to dose honey bees in both the range-finding and definitive bioassays, because solutions >200 mg/ml clogged the microsyringe, making it impossible to deliver accurate doses of OA. The results for the definitive bioassay are summarized in Table 1. Only the 10-μl acetone control group was used for Probit analysis of the definitive bioassay data, because the 10-μl acetone control had slightly more mortality than the dry control group. The natural mortality for the definitive bioassay was 4.0 ± 2.5% after 48 h. We were unable to calculate a conﬁdence interval for the
20-ml scintillation vial treated with OA was based on the 100 and 200/\textit{H9262} 400 estimated 72-h LD50 for honey bees was 194.89 clean glass petri dishes at 32.5 solutions of OA. The mites were then transferred to mites collected from brood were exposed to OA for 4 h results indicate that the 24-h LC50 for phoretic mites is 5.12/\textit{H9262} 1.9/\textit{H9262} 72-h LD50, because all bees in the 2,000, 1,600, 800, and 400 \(\mu\)g of OA per bee treatments died after 72 h. The estimated 72-h LD50 for honey bees was 194.89 \(\mu\)g per bee, based on the 100 and 200 \(\mu\)g of OA per bee treatments.

Discussion

Milani (2001) is the only report that quantifies the toxicity of OA to \textit{V. destructor} collected from bee brood, where the 24-h median lethal density (OA density expected to cause 50% mortality) for \textit{V. destructor} collected from bee pupae with white eyes was 1.9 \(\mu\)g/cm\(^2\) (95% fiducial limits = 1.49–2.36). The mites collected from brood were exposed to OA for 4 h by placing them on glass disks that were sprayed with solutions of OA. The mites were then transferred to clean glass petri dishes at 32.5°C and 75% RH. Our results indicate that the 24-h LC50 for phoretic mites is 5.12 \(\mu\)g per vial. By assuming the area of a single 20-ml scintillation vial treated with OA was \(\approx\)20 cm\(^2\), the 24-h median lethal density in our study was calculated to be 0.26 \(\mu\)g/cm\(^2\). One likely reason for the apparent higher toxicity in our study is that Milani exposed mites to OA for 4 h versus 24 h in our bioassays. Furthermore, the previous study used mites collected from brood as experimental material, and we collected mites from adult bees. Differences between mites collected from adults versus brood could have also affected bioassay results. However, we think it is preferable to use phoretic mites for conducting OA bioassays, because OA does not kill mites in brood when applied to a hive.

The mode of action for OA is not completely understood. Our results suggest that OA may exhibit its lethal effects on mites via contact. However, we cannot rule out that some mite mortality was caused by exposure to OA vapors. We expect that mite mortality resulting from exposure to OA vapors was minimal because OA has a low volatility at room temperature (vapor pressure <0.001 mmHg at 25°C; melting point of 101–102°C) (Merck Index 1996). Charrière and Imdorf (2002) reported that OA mixed in 2:1 sugar water solution adheres better to bees, thus increasing mite exposure to OA.

The 400, 800, 1,600, and 2,000 \(\mu\)g per bee doses applied in the definitive bioassay completely covered the bees’ abdomen with OA crystals. Furthermore, the 2,000 \(\mu\)g per bee dose that was delivered in 10 \(\mu\)l of acetone completely soaked the bees with some runoff. Our results indicate that it is nearly impossible to kill 100% of a test population of adult bees in 24 h by topically applying OA in acetone. We conclude that OA has a relatively low acute toxicity to honey bees. There are several accounts in the literature of increased adult bee mortality as a result of OA application within hives (Charrière and Imdorf 2002, Imdorf and Charrière 1995), suggesting that OA may not exhibit its lethal effect on honey bees until >24 h after exposure.

A typical honey bee colony in the North Central region of the United States has a November adult bee population of 35,000 bees (unpublished data). According to the Canadian protocol (Special Supplement 2005), a beekeeper would apply a maximum of 2 g of OA per hive for \textit{V. destructor} treatment (trickle or vaporizer methods). The resulting dose of OA per bee would have a maximum value of 57.1 \(\mu\)g per bee (2,000,000 \(\mu\)g of OA per 35,000 bees). Results from acute exposure bioassays suggest that 57.1 \(\mu\)g per bee is considerably below the 48-h LD10 (176.68 \(\mu\)g per bee). Our results showed that all bees dosed with 2,000, 1,600, 800, or 400 \(\mu\)g of OA per bee died after 72 h. Bees subjected to the 100 \(\mu\)g of OA per bee dose survived longer than 72 h and had mortality similar to the control treatment. Our results roughly coincide with the recommended dose of OA per hive (\(\geq 2\) g), because doses \(\leq 100\) \(\mu\)g of OA per bee in our laboratory bioassays did not cause significant mortality.

Our data are significant because they quantify the dosage–mortality relationships for populations of both mites and bees that have not been exposed to OA. Our data will facilitate future comparisons of toxicity if mite resistance to OA becomes evident. Furthermore, these basic toxicological properties of the compound will help guide scientists in developing techniques for delivering optimum dosages to the parasite and its host.

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### Table 1. Honey bee mortality responses to OA dihydrate when topically applied to bee abdomens in acetone and \textit{V. destructor} mortality responses in glass-vial residual bioassays

<table>
<thead>
<tr>
<th>Mortality</th>
<th>(n)</th>
<th>Slope ± SE</th>
<th>LD10 (95% CL)</th>
<th>LD50 (95% CL)</th>
<th>LD90 (95% CL)</th>
<th>(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Honey bee</td>
<td>24 h</td>
<td>280</td>
<td>2.87 ± 0.54</td>
<td>564.05 (95.22–577.92)</td>
<td>1,575.65 (1,087.44–2,962.72)</td>
<td>4,402.6 (2,541.78–8,485.75)</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>280</td>
<td>3.96 ± 0.54</td>
<td>176.68 (120.36–225.544)</td>
<td>372.01 (306.78–439.88)</td>
<td>783.27 (643.5–1,042.56)</td>
</tr>
<tr>
<td>Mite</td>
<td>24 h</td>
<td>320</td>
<td>2.28 ± 0.35</td>
<td>1.4 (0.63–2.26)</td>
<td>5.12 (3.47–7.0)</td>
<td>18.69 (13.14–31.7)</td>
</tr>
</tbody>
</table>

LD values for honey bees are in micrograms per bee; LD values for mites are in micrograms per 20-ml vial.
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