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Bee-to-bee contact drives oxalic acid distribution in honey bee colonies

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Original article

Bee-to-bee contact drives oxalic acid distribution in honey bee colonies*

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Abstract – Nine divided hives were constructed to study the distribution of oxalic acid (OA). Experimental colonies were split into two equal, queenright sections with one of three divider types. The first divider allowed trophallaxis to occur between adult bees on each side, but did not allow bee-to-bee contact. The second divider did not allow trophallaxis or bee-to-bee contact. The third divider allowed both bee-to-bee contact and trophallaxis between the two sides. All three dividers allowed gas exchange of volatile materials. The objective was to investigate factors that contribute to the distribution of OA in a hive by monitoring *Varroa destructor* mortality. Forty mL of a 3.5% OA sugar water solution was trickled on one side of the divider. Sticky boards were used to quantify mite fall before, during, and after OA treatment on both treated and untreated sides. Trophallactic interactions and fumigation did not significantly influence the distribution of OA. Bee-to-bee contact was the primary route for OA distribution.

***Varroa destructor* / *Apis mellifera* / oxalic acid / mode of action / distribution**

1. INTRODUCTION

Oxalic acid (OA) is widely used for controlling *Varroa destructor* in Europe and Canada due to its high efficacy (> 90%) and low risk of hive contamination (Charrière and Imdorf, 2002; Special Supplement, 2005). Its registration is pending in the United States. OA is applied to colonies by spraying or trickling a solution of OA and sugar water over the bees or by evaporating crystals with heat. Most research reviewed by Nanetti et al. (2003) and Rademacher and Harz (2006) found that a single autumn trickle treatment with a 3.0% OA sugar water solution (1:1 by weight) provided an efficacy of greater than 90% in Central Europe. Although OA provides effective control of *V. destructor*, its mode of action and distribution in honey bee colonies are unknown.

Aliano et al. (2006) hypothesized that OA may kill *Varroa destructo*r mites via contact. They reported that the 24 hour LC_{50} (95% CL) for phoretic mites was 5.12 (3.5 to 7.0) μg of OA per 20-mL vial. Milani (2001) quantified the toxicity of OA to *V. destructor* collected from bee brood. Milani reported that the 24 hour LD_{50} (95% CL) (median lethal density) for mites collected from brood was 1.9 (1.49 to 2.36) μg/cm^2. The results from Aliano et al. (2006) and Milani (2001) suggest that OA has a high acute toxicity to mites. Aliano et al. (2006) indicate that the high acute toxicity of OA to *V. destructor* in glass-vial residual bioassays suggests that OA readily kills mites that come in physical contact with the crystals. The authors concede that some mite mortality could have been caused by exposure to OA vapors within the scintillation vials.

The objective of the current study was to identify factors that contribute to the distribution of OA in a hive and to test the Aliano et al. (2006) hypothesis that OA kills mites via contact. The importance of fumigation, trophallaxis, and direct contact when trickling OA were evaluated. The results will give

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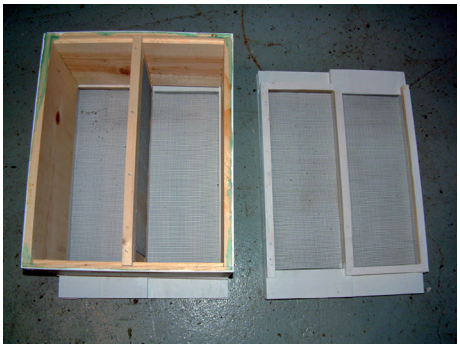

Figure 1. Left: split-unit Langstroth hive with single-screen divider. Right: screened bottom board with opposing entrances.

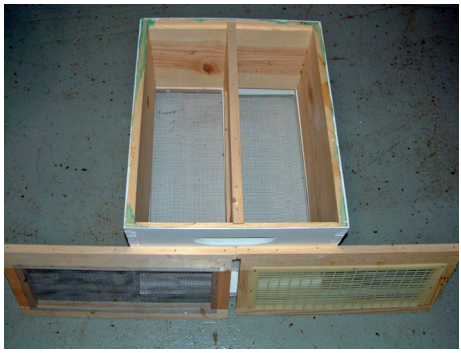

Figure 2. Left to right: double screen divider and queen excluder divider.

beekeepers and researchers insight as to how OA is distributed in hives. Our results provide guidance for selecting application techniques that maximize the efficacy of OA.

2. MATERIALS AND METHODS

2.1. Construction of divided (split-unit) hives

We designed and built 9 divided single-story Langstroth hives in June, 2005. Our hives resembled standard, single story Langstroth beehives. We modified the boxes by splitting them into two equal sections that held 4 frames each (Fig. 1). The sections were separated using one of 3 different dividers. All dividers had a 2 × 46.5 cm wooden frame that formed bee-tight seals between the sides of the hive body, the inner cover, and the bottom board. The first divider (single-screen divider) had a 585 cm^2 area in its center made from 8-mesh screen and it allowed trophallaxis and gas exchange between bees on the two sides. The second divider (double-screen divider) had a 585 cm^2 area in its center made from two pieces of 8-mesh screen that were separated by a two cm gap. It allowed gas exchange, but did not allow trophallaxis between the two sides. The third divider (queen excluder divider) had a 585 cm^2 area in its center made from plastic queen excluder that allowed worker bees to move freely between the two sides. It allowed trophallaxis, gas exchange, and bee-to-bee contact between the two sides. The three dividers described above are shown in Figure 2.

All divided colonies had a separate entrance for each side. The entrances faced opposing directions to minimize the drift of adult bees from side-to-side. In addition, the bottom board was fitted with 8-mesh screen that allowed mites to fall onto a sticky board placed below the screen. This allowed us to independently monitor mite fall on each side of the divider (Fig. 1).

Our divided hives were designed to allow us to examine the distribution of OA by treating one side and monitoring the resulting mite fall in both the treated and untreated sections. We expected similar mite fall on the sides that were treated with OA regardless of divider. Our intention was to correlate mite fall on the untreated section with divider type. The design of our dividers allowed us to restrict the amount of adult bee interaction between each half-unit and ranged from complete isolation (double-screen divider) to minimal isolation (queen excluder divider) as described above.

2.2. Stocking of hives

We stocked the 9 divided hives by splitting *V. destructor*-infested colonies from an apiary located at the University of Nebraska Agricultural Research and Development Center on June 29, 2005. The apiary was composed of a mixture of Carniolan and Italian honey bees (*Apis mellifera* L.). At this time, all hives were given a solid-wood divider. Each side of the divided hive was furnished with a frame of capped brood, a frame of honey, a frame of pollen, and an empty frame with foundation. This resulted in 4 frames for each side and 8 frames for the entire divided Langstroth hive. Adult bees were transferred to the units directly on the combs from which the splits were made. The hives were immediately sealed and moved approximately 56 km to the University of Nebraska-Lincoln East Campus. A 15 day

old queen cell was placed in each side of the divided colonies the following day (June 30). The hives were then left untouched for approximately two weeks. This period allowed mites to emerge from brood cells and gave the virgin queens time to mate and begin laying eggs.

We randomly assigned the 9 hives to three treatment groups. Three hives were assigned to each of three treatment groups (single-screen divider, double-screen divider, and queen excluder divider). We removed the solid-wood dividers that were used when the units were stocked and replaced them with the appropriate dividers listed above. We also verified that each side of the divided colony was queenright and that sealed brood was not present. Only divided hives that had successfully reared a queen on each side were included in this experiment. We used queen cells to make each side queenright resulting in hives devoid of capped brood during treatment. This ensured that all mites present in the hives were phoretic on adult bees and vulnerable to OA treatment.

2.3. Treatment and data collection

We replaced the sticky boards prior to OA application (July 15). One side of the 9 divided hives was treated with 40 mL of a 3.5% OA sugar water solution (sugar:water) (1:1) (w:w). The OA solution was trickled from above the frames between each occupied bee-way using a 100 mL syringe and an effort was made to maximize contact with the adult bee population. This dose was chosen based on a review article for treating colonies with minimal capped brood (Rademacher and Harz, 2006).

The sticky boards were replaced and mite fall counted at 2, 4, and 6 days post-treatment (July 17, 19, and 21). A Checkmite® strip was placed in each half-hive to quantify remaining mites (July 21) (the experimental mite population had not previously exhibited coumaphos resistance). Sticky boards were replaced every 48 hours until no mites were detectable (July 23 and 25). Use of the Checkmite® strips allowed us to quantify the total number of mites in each hive prior to OA application. We added the total number of mites recovered 2, 4, and 6 days after OA treatment to the number of mites recovered after Checkmite® strips were placed in the hives. This enabled us to calculate the post-treatment percentage mite fall at 2, 4, and 6 days.

2.4. Replication

We replicated the entire experiment to increase the power of our tests (September 2005). The materials and methods were similar to those listed in sections 2.2 and 2.3 above. The only difference was that queen cells were not added to the hives. Instead, we allowed the bees to rear a queen from a small patch of eggs that was deliberately left when the units were stocked. Like adding queen cells, allowing the units to rear their own queen ensured that the hives would be void of capped brood during experimentation.

2.5. Experimental design and statistical analysis

We used a split-plot experimental design to analyze our data. The whole plot factor was divider type (single-screen, double-screen, and queen excluder) and the whole plot unit was the entire hive. The split-plot factor was treatment with OA (treated and untreated) and the split-plot unit was a half hive. We used the percentage reduction in varroa infestation 2, 4, and 6 days post-treatment as our response variable. We blocked by the month in which the experiment was conducted (July and September) to account for variance in the total mite infestation between the two replicates.

We analyzed the data using PROC MIXED (SAS Institute, 2003) and separated means using a paired *t*-test ($\alpha = 0.05$). We assumed random blocks, although the assumption of fixed blocks did not change the results. We used the Kenwood-Rogers degrees of freedom adjustment. We used PROC UNIVARIATE and PROC GPLOT (SAS Institute, 2003) to verify our assumptions of normality and constant variance.

3. RESULTS

3.1. Forty eight hour pre-treatment mite fall

The 48 h pre-treatment mite fall was 31.2 ± 4.1 mites per split-unit hive in the July replicate ($n = 18$) and 45.3 ± 7.6 mites per split-unit hive in the September replicate ($n = 18$). The pre-treatment mite fall was not significantly different for the sides scheduled to receive OA versus the sides scheduled to be left untreated for either replicate ($t = 0.22$, $df = 32$, $P = 0.8236$).

3.2. Total mite infestation

The total number of mites recovered per split-unit hive was 389 ± 52 mites ($n =$

Table I. *F*tests for RCBD split-plot in time.

effect	numerator *df*	denominator *df*	*F*	*P*
divider	2	14	5.9	0.0142
treatment	1	75	853.8	0.0001
divider × treatment	2	75	75.8	0.0001
time	2	75	38.1	0.0001
divider × time	4	75	0.9	0.4567
treatment × time	2	75	0.1	0.8994
divider × treatment × time	4	75	0.2	0.9341

18) for the July replicate. The total number of mites recovered per split-unit hive was 665 ± 52 mites (n = 18) for the September replicate. The total varroa infestation in the September replicate was 276±73 mites greater per split-unit hive than the July replicate (t = 3.76, df = 34, P = 0.0006).

3.3. Randomized Complete Block Design (RCBD) split-plot in time

Our assumptions of normality and constant variance were met. We used the Shapiro-Wilk test in the UNIVARIATE procedure of SAS to verify normality. The Shapiro-Wilk test indicated that our data was normal (P = 0.5722). In addition, a symmetric box-plot and a straight-lined normal probability plot confirmed normality. A plot of the residual versus the predicted values revealed no obvious patterns and was indicative of data that had constant variance.

In total, there were 18 split-unit hives that were sampled at 2, 4, and 6 days post-treatment. Eighteen hives multiplied by 3 sample intervals equals 54 observations per replicate. Fifty four observations in the July replicate plus 54 observations in the September replicate sum to 108 total observations. The response variable was percentage reduction in varroa infestation. See Table I for a summary of the F tests for the RCBD split-plot in time effects.

There was significant divider × treatment interaction (P = 0.0001). The factor 'time' was not part of this interaction so we analyzed the main effect for time. The time effect was significant (P = 0.0001). There was a 43.1 ± 3.6% (n = 36), 51.4 ± 3.6% (n = 36) and 58.5 ± 3.6% (n = 36) reduction in varroa infestation at 2, 4, and 6 days after OA application. The above means represent the average mite fall per split-unit hive regardless of divider type or treatment.

Significantly more mites fell by day 6 than by days 2 or 4. Explicitly, 8.4 ± 1.8% more mites fell by day 4 versus day 2 (t = 4.73, df = 75, P = 0.0001), 7.1 ± 1.8% more mites fell by day 6 versus day 4 (t = 3.98, df = 75, P = 0.0001), and 15.4 ± 1.8% more mites fell by day six versus day 2 (t = 8.71, df = 75, P = 0.0001).

3.4. RCBD split-plot on 6 day percentage mite fall

The analysis of the RCBD split-plot in time confirmed that it was appropriate to only model the 6 day percentage mite fall because more mites fell by day 6 than days 2 and 4. To simplify our model, we removed the time factor and used 6 day percentage mite fall as our sole response variable in our subsequent data analysis. This reduced the total number of observations from 108 to 36 (108 total observations/3 time intervals = 36 observations for 6 day percentage mite fall). See Table II for a summary of the F tests for the RCBD split-plot on 6 day percentage mite fall.

There was significant divider × treatment interaction (P = 0.0001). We did not consider the main effects of divider and treatment because of the significant interaction term. Rather, we analyzed the simple effects to draw

Table II. *F*tests for RCBD split-plot on 6 day percentage mite fall.

effect	numerator *df*	denominator *df*	*F*	*P*
divider	2	14	6.8	0.0086
treatment	1	15	179.6	0.0001
divider × treatment	2	15	18.0	0.0001

Table III. Percentage reduction in varroa infestation 6 days post-treatment. Estimates with different letters indicate significant differences (*t*-test, $\alpha = 0.05$).

divider type / treatment	estimate ± standard error	n
single screen / oa* treated side	73.3 ± 7.5 a	6
double screen / oa treated side	84.8 ± 7.5 a	6
queen excluder / oa treated side	80.5 ± 7.5 a	6
single screen / untreated side	22.6 ± 7.5 b	6
double screen / untreated side	25.2 ± 7.5 b	6
queen excluder / untreated side	64.6 ± 7.5 c	6

* oxalic acid.

conclusions about these two factors. Table III is a summary of the six treatment means reported as percentage reduction in varroa infestation. Treatment combinations in the divider/treatment column with 'OA treated side' indicate that OA was applied. Treatment combinations in the divider/treatment column with 'untreated side' indicate that OA was not applied.

The sides that were treated with OA had significantly more mite fall than the untreated sides for all 3 dividers. When only considering the units with single-screen divider, sides that were treated with OA had 50.7 ± 5.4% greater mite fall than the sides left untreated ($t = 9.33$, $df = 15$, $P = 0.0001$). When only considering the units with double-screen divider, sides that were treated with OA had 59.6 ± 5.4% greater mite fall than the sides left untreated ($t = 10.96$, $df = 15$, $P = 0.0001$). When only considering the units with queen excluder divider, sides that were treated with OA had 15.9 ± 5.4% greater mite fall than the sides left untreated ($t = 2.92$, $df = 15$, $P = 0.0105$).

There was no difference in the percentage mite fall on the sides that were treated with OA for all three dividers. When only considering the sides that were treated with OA; units with double-screen dividers had 4.4 ± 7.8% greater mite fall than units with queen excluder dividers ($t = 0.55$, $df = 22.2$, $P = 0.5849$), units with double-screen dividers had 11.5 ± 7.8% greater mite fall than units with single-screen dividers ($t = 1.46$, $df = 22.2$, $P = 0.1579$), and units with queen excluder dividers had 7.1 ± 7.8% greater mite fall than units with single-screen dividers ($t = 0.91$, $df = 22.2$, $P = 0.3742$).

When only considering the untreated sides, units with the queen excluder divider had significantly more mite fall than units containing either single- or double-screen dividers. When only considering the untreated sides, units with the queen excluder divider had 39.3 ± 7.8% greater mite fall than units containing double-screen dividers ($t = 5.01$, $df = 22.2$, $P = 0.0001$) and units with the queen excluder divider had 42.0 ± 7.8% greater mite fall than units containing single-screen dividers ($t = 5.35$, $df = 22.2$, $P = 0.0001$). The percentage mite fall on the untreated sides was not significantly different for units containing the single-screen versus the double-screen dividers ($t = 0.34$, $df = 22.2$, $P = 0.7403$).

4. DISCUSSION

As expected, the percentage mite reduction was not significantly different on the sides of

the divided hives that were treated with OA regardless of divider type. Our intention was to correlate mite fall on the untreated side with divider type. Divided hives with single-screen and double-screen dividers averaged 23 and 25% mite fall on their untreated sides after six days, respectively. Trophallactic interactions and fumigation did not significantly influence the distribution of OA as single-screen and double-screen divided hives had similar mite fall on their untreated sides. As Table III illustrates, bee-to-bee contact was the primary route for OA distribution because divided hives with queen excluders had significantly more mite fall (65%) on their untreated sides than divided hives with single-screen or double-screen dividers. Only the queen excluder divider permitted worker bees to move freely and allowed bee-to-bee contact between the two sides. We accept the Aliano et al. (2006) hypothesis that OA kills mites via contact.

Significantly more mites fell six days after OA application than 2 or 4 days after OA application. This statistic may be interpreted several ways. One interpretation is that OA has residual activity against varroa for at least six days post-treatment. Charrière et al. (2004) and Gregorc and Planinc (2004) report that mite fall can occur over a 3 week period in hives treated with OA. Another interpretation is that a portion of the varroa mites exposed to OA experience a drawn-out death. Aliano et al. (2006) and Milani (2001) demonstrate that OA has a high acute toxicity to mites in laboratory bioassays. These studies do not quantify the chronic toxicity of OA to *V. destructor* because of the impossibility of sustaining mite populations for long periods of time away from their honey bee hosts. Perhaps the chronic toxicity of OA for phoretic mites in the hive environment is significantly less than the acute toxicity reported by Aliano et al. (2006) and Milani (2001).

One important assumption of our experiment was that the single-screen divider allowed trophallaxis to occur between adult bees on each side. This assumption held true throughout experimentation as we observed adult bees performing proboscis extensions and trophallactically interacting between the single-screen dividers. The role of trophallaxis in the distribution of Perizin (coumaphos) in honey bee colonies was investigated by van Buren et al. (1992). Van Buren et al. (1992) divided hives into three compartments with screens and traced the amount of coumaphos transferred between the sections via trophallaxis. Although trophallactic interactions were of minor importance in the distribution of coumaphos, the authors indicate that trophallaxis was occurring between the screened sections of the hive.

Anecdotal observations from beekeepers suggest that adult honey bees will ingest sugar water feed containing OA. We noticed small, pea-size pools of the OA sugar water solution on the top bars of several hives up to 6 days after OA application. We did not observe ingestion of the OA solution by adult bees and the pools eventually evaporated. If the anecdotal observation that bees will ingest sugar water containing OA is true, our results suggest that the concentration must be lower than 3.5% OA by weight. Our results only apply to the trickle method with a 3.5% OA sugar water solution (1:1) (w:w). The distribution of OA in honey bee colonies when the vaporizer method is used was not tested in our study. Our results give beekeepers and researchers insight as to how OA is distributed in hives and provide guidance for selecting application techniques that maximize the efficacy of OA.

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Les contacts entre abeilles sont responsables de la répartition de l'acide oxalique dans les colonies d'abeilles.

***Apis mellifera* / *Varroa destructor* / acide oxalique / mode d'action / répartition**

Zusammenfassung – Biene zu Biene Kontakt ist verantwortlich für die Verbreitung von Oxalsäure in Honigbienenvölkern. Oxalsäure (OS) wird intensiv zur Bekämpfung von *Varroa destructor* in Europa und Kanada eingesetzt, zum einen wegen ihres hohen Wirkungsgrades (> 90 %) und zum anderen wegen der geringen Rückstandsgefahr (Charrière and Imdorf, 2002; Special Supplement, 2005). In den Vereinigten Staaten läuft das Zulassungsverfahren. OS wird im Bienenvolk entweder als Lösung mit Zuckerwasser auf die Bienen versprüht oder geträufelt oder aber als kleinste Kristalle bei hoher Hitze verdampft. Obwohl OS eine effektive Bekämpfungsmöglichkeit gegenüber *V. destructor* darstellt, sind die Wirkungsweise und die Verteilung im Bienenvolk ungeklärt.

Mit dieser Studie sollten durch die Bestimmung der Mortalität von *V. destructor* unter verschiedenen Bedingungen Faktoren analysiert werden, die für die Verbreitung der OS innerhalb des Bienenstockes verantwortlich sind.

Dabei wurde die Bedeutung von Gasaustausch, Trophallaxis und direktem Kontakt beim Träufeln der OS erfasst. Hierfür wurden neun Bienenkästen, die jeweils in zwei Einheiten unterteilt waren, verwendet. Die Testvölker wurden in zwei gleich große und weiselrichtige Einheiten unterteilt und durch einen von drei unterschiedlichen Trennschieden voneinander getrennt. Der erste Typ des Trennschiedes erlaubte Trophallaxis zwischen den Bienen der beiden Einheiten, aber keinen direkten Kontakt zwischen den Bienen der beiden Einheiten. Der zweite Typ erlaubte weder Trophallaxis noch direkten Kontakt. Der dritte erlaubte Trophallaxis und direkten Kontakt zwischen den Bienen beider Seiten. Bei allen drei Schieden war Gasaustausch möglich. 40 mL einer 3,5 % OS-Zuckerwasser-Lösung wurde dann auf die Bienen einer Seite des unterteilten Volkes geträufelt. Auf Bodeneinlagen, die mit Klebstoff versehen waren, wurde der Milbenabfall vor und nach Behandlung sowohl im behandelten als auch im unbehandelten Teil erfasst.

In Tabelle III sind die Mittelwerte der jeweils sechs Behandlungen als Prozent der Abnahme des Varroa-Befalls zusammengefasst dargestellt. In der „divider/treatment"-Säule ist aufgeführt, ob bei der ausgewerteten Einheit OS direkt angewendet wurde („OA treated side") oder nicht („untreated side").

Es gab keinen Unterschied im prozentualen Milbenfall bei den Einheiten, die mit OS behandelt wurden unabhängig vom Typ des Trennschiedes. Bei den nicht behandelten Einheiten hatten die mit Königinnenabsperrgitter abgetrennten Testvölker einen signifikant höheren Milbenfall als solche mit Einfach- oder Doppelgitter getrennte Einheiten. Trophallaxis und Gasaustausch beeinflusste die Verteilung der OS nicht signifikant.

Lediglich das Trennschied aus Königinnenabsperrgitter erlaubte den Arbeiterinnen freie Bewegung zwischen den beiden Einheiten und damit Biene zu Biene Kontakt zwischen behandelter und unbehandelter Seite. Tabelle III zeigt, dass Biene zu Biene Kontakt der entscheidende Weg für die Verbreitung der OS ist, da die mit Absperrgitter geteilten Testvölker einen signifikant höheren Milbenfall aufwiesen (65%) als Testvölker, die durch die zwei unterschiedlichen Gitter voneinander getrennt waren. Unsere Ergebnisse sollen Imkern und Bienenwissenschaftlern ein besseres Verständnis über die Verteilung von OS im Bienenvolk geben und dazu beitragen, Applikationsformen mit einer noch effektiveren Verteilung der OS zu entwickeln.

***Varroa destructor* / *Apis mellifera* / Oxalsäure / Wirkungsweise / Verteilung**

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