Short-Term Fumigation of Honey Bee (Hymenoptera: Apidae) Colonies with Formic and Acetic Acids for the Control of *Varroa destructor* (Acari: Varroidae)

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ABSTRACT Controlling populations of varroa mites is crucial for the survival of the beekeeping industry. Many treatments exist, and all are designed to kill mites on adult bees. Because the majority of mites are found under capped brood, most treatments are designed to deliver active ingredients over an extended period to control mites on adult bees, as developing bees and mites emerge. In this study, a 17-h application of 50% formic acid effectively killed mites in capped worker brood and on adult bees without harming queens or uncapped brood. Neither acetic acid nor a combined treatment of formic and acetic acids applied to the West Virginia formic acid fumigator was as effective as formic acid alone in controlling varroa mites. In addition, none of the treatments tested in late summer had an effect on the late-season prevalence of deformed wing virus. The short-term formic acid treatment killed >60% of varroa mites in capped worker brood; thus, it is a promising tool for beekeepers, especially when such treatments are necessary during the nectar flow.

KEY WORDS varroa mites, formic acid, acetic acid, fumigation, deformed wing virus

The varroa mite, *Varroa destructor* Anderson & Trueman, an ectoparasitic mite of honey bees, *Apis mellifera* L., has caused widespread damage to the North American beekeeping industry (deJong 1997). Mite-infested colonies die from parasitism within a few years unless mite control methods are implemented (Fries et al. 1994). However, little experimental data are available to substantiate the assumption that varroa mites directly cause colony mortality (Ball 1993). Although mites do damage their parasitized hosts, a growing body of evidence suggests that the viruses vectored and/or activated by mites have a larger negative impact on their hosts than the mites themselves. One virus commonly associated with varroa mites is deformed wing virus (DWV). Before the widespread dispersal of varroa mites, this virus was considered benign. However, in association with varroa mites, DWV causes bee mortality and can lead to colony death (Bowen-Walker et al. 1999; Nordström 2003; Chen et al. 2004; Shen et al. 2005; Yang and Cox-Foster 2005, 2007). Given the combined impact of mites and viruses, effective varroa mite control strategies should not only consider the impact on mite populations but also the effect on virus levels.

Formic acid (FA) fumigation has received considerable attention as a mite control product (Calderone and Nasr 1999, Kochansky and Shimanuki 1999, Cal-

derone 2000, Hood and McCreadie 2001, Underwood and Currie 2004). One major advantage of FA fumigation is that it provides control for other honey bee parasites, including the honey bee tracheal mite, *Acarapis woodi* (Rennie) (Wilson et al. 1993, vanEngelsdorp and Otis 2001), *Tropilaelaps clareae* Delfinado et Baker (Hoppe et al. 1989, Sharma et al. 2003), and possibly nosema disease (*Nosema apis* Zander) (Hoppe et al. 1989, Sharma et al. 2003, Underwood and Currie 2004). However, the impact FA fumigation has on virus load in a colony has not been documented.

To prevent contamination of harvested honey, product labeling of all chemical mite controls restricts application to colonies that have had their honey supers removed. Thus, beekeepers with harmful mite levels during the nectar flow are forced to decide between delaying treatment or removing the honey crop prematurely to treat immediately. Removing honey stores for long periods during the late-season nectar flow is not a viable option because incoming nectar is then ripened and stored as honey in the brood chamber, restricting brood production, and, consequently, the production of "winter bees." However, if treatment is delayed, colonies may die or become severely weakened and they are unlikely to survive the winter (Amdam et al. 2004) because of mite parasitism and increased levels of varroa mite-vectored viruses (Ball 1997). Developing a treatment that could be applied during the nectar flow for a short duration would allow beekeepers to treat their colonies in a timely manner without suffering the negative

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impact of removing honey stores for a long period or delaying treatment until the honey flow ends.

All registered chemical mite controls kill mites on adult bees. However, when brood is present in colonies, a majority of the mites are beneath the cappings of older brood and reproducing (Fries et al. 1994). Therefore, the effectiveness of a chemical control to date is dependent on ensuring its presence in the colony as mites emerge with the newly emerging adult bees. This is achieved either through multiple applications of the product or devising a continuous release of the product over an extended period. Thus, a control that kills mites under the cappings in addition to those on adult bees would be extremely desirable and would reduce the amount of time to achieve effective control. Studies have shown that higher concentrations of formic acid can kill mites under cappings (Fries 1989, Calis et al. 1998, Calderon et al. 2000, Amrine et al. 2006). Unfortunately, these studies did not examine the effects on brood, had highly variable results, and they did not quantify effects on mite mortality.

The primary objective of this study was to test the ability of formic and acetic acids, used alone or in combination as 17-h applications to kill varroa mites both in capped brood and on adult bees. In addition, the effects of treatments on honey bee brood, queen survival, and prevalence of deformed wing virus were determined. Treatments were applied during the late-season nectar flow.

Materials and Methods

Apiary and Colony Selection. Three apiaries were selected for this study, one in Harrisburg, PA, with 16 colonies (PDA; latitude 40.28894, longitude −76.87037) and two in Berks County, PA, with 20 colonies each (Grange; latitude 40.442390, longitude −76.11552; Way Har; latitude 40.47695, longitude −76.14283). All colonies had brood nests composed of one standard deep and one shallow hive body. Each brood box contained 10 frames, had approximately equal numbers of bees, and had a laying queen.

Pretreatment Assessment. On 20, 22, and 23 August 2004 sticky boards (IPM Varroa boards, Great Lakes IPM Inc., Vestaburg, MI) coated with petroleum jelly were placed under plastic screens (Dadant & Sons, Ltd., Hamilton, IL) and inserted onto the bottom boards of study colonies. Sticky boards were removed 3 d later and average daily mite drop was quantified (Ostiguy and Sammataro 2000).

Colonies were opened on 23, 25, and 26 August 2004. The total amount of capped brood in colonies was quantified using a standard-size wooden frame without foundation that had been laced with string to create a 10-section grid (8.5 by 10.25 cm per section). The percentage of each section containing capped worker and drone brood was estimated to the nearest 10% and converted into the total number of frames of capped brood (after Fries et al. 1994). While quantifying brood, the presence of the colony's queen was noted.

Varroa mite prevalence (proportion of capped cells containing mites) and intensity (number of mites per infested brood cell) (Margolis et al. 1982) in capped brood were determined by counting the number of live mature female mites (Calderon et al. 2000) contained in capped drone and worker cells. When possible, 20 drone cells and 100 worker cells per colony were examined. The capped worker brood was always taken from a central frame found in the lower brood chamber. To determine viability, mites were prodded with forceps and those that moved were considered alive (Calderon et al. 2000). A sample of 118 ml (one-half cup or ≈320 bees) of bees was collected from the brood nest of each colony and placed in alcohol for later quantification of mean varroa mite abundance on adult bees (number of mites per 100 bees) (Margolis et al. 1982, Shimanuki and Knox 2000, Rinderer et al. 2004). In one apiary (PDA), 20 adult worker bees were collected from brood frames and individually placed into 1.5-ml microcentrifuge tubes. These samples were stored in vaporous liquid nitrogen, transferred into 1 ml of TRIzol reagent (Invitrogen, Carlsbad, CA), and stored at −80°C until DWV prevalence could be determined as described below.

Treatment. Colonies in each apiary were divided into four groups, with the colonies in each group having approximately the same amount of brood. Each of the resulting groups was randomly assigned to one of four treatments: 1) W, 100 ml of distilled water; 2) FA, 75 ml of 50% formic acid; 3) AA, 50 ml of 50% acetic acid; or 4) F + A, formic and acetic acids (75 ml of 50% FA + 50 ml of 50% AA) applied at the same time into a colony on separate fume boards. The F + A treatment was not applied in the PDA apiary.

Immediately before treatment, a sticky board was inserted into each colony and hive entrances were reduced to 5.1 by 2.1 cm by using duct tape. Any holes in or between hive components also were sealed using duct tape. Treatments were applied at ≈1600 hours on 23, 25, and 26 August 2004 by using West Virginia formic acid fumigation boards (Amrine et al. 2006). Amrine et al. (2006) provide complete details on the specifications of this fumigation board.

Ambient temperature and humidity measurements for the 17-h treatment period were obtained from the weather stations nearest to each apiary (site ID DEPHAR for the PDA apiary, and site ID DOT1011 for the Grange and Way Har apiaries; Climatologist 2005).

Posttreatment Assessment. At ≈0900 hours the morning after treatment application, the fumigation boards and sticky boards were removed. Varroa mite prevalence, intensity, and viability in drone and worker brood were again assessed as described above. However, in addition to assessing viability in a frame from the center of the bottom brood chamber, the brood on a shallow frame in the center of the upper brood chamber and/or on a frame on an outer edge of the bottom brood chamber was examined in a subset of FA-fumigated hives in the Grange and Way Har apiaries.

One week after treatment, capped brood area was again quantified as described above. The condition of

each colony's queen was also noted and those colonies found to be queenless were removed from the study. On 15 and 16 September, mite counts were again taken by sticky boards placed into colonies for 4 d. A sample of 118 ml (one-half cup or ≈320 bees) of bees was collected from each colony and placed in alcohol for quantification of varroa mite mean abundance. Individual bees were again collected from colonies in the PDA apiary for virus analysis.

Analysis of DWV Virus with Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Total RNA was isolated from each worker bee by homogenization in 500 μl of TRIzol reagent (Invitrogen), and treatment with the RQ1 RNase-Free DNase (Promega, Madison, WI) according to the manufacturer's specifications. RNA concentration was determined spectrophotometrically (SmartSpec 300, Bio-Rad, Hercules, CA). Five micrograms of total RNA from each sample was used to make cDNA with the Moloney murine leukemia virus reverse transcriptase kit (Promega) according to the manufacturer's specifications. Primers were designed for their specificity to DWV (forward, 5′-ACGACACAA CATCCTGTA-G-3′ and reverse, 5′-TAAACTAGGTTGGACTGGA-A-3′) based on the DWV genomic sequence (GenBank accession no. NC_004830), which amplify a 621-bp amplicon. Honey bee actin gene (GenBank accession no. BI_504901) was used as an internal control for the RT-PCR. β-Actin primers (forward, 5′-ATGAAGATCCTTACAGAAAG-3′ and reverse, 5′-TCTTGTTTAGAGATCCACAT-3′) amplify an amplicon of 514 bp. A negative control lacking template cDNA was included each time. The PCR reaction was carried out in 1X PCR buffer (50 mM KCl, 10 mM Tris-HCl, and 0.1% Triton X-100) containing 0.2 mM dNTPs, 2.0 mM MgCl$_2$, 2 ng/μl each primer, 0.05 U/μl *Taq*DNA polymerase (Promega), and 1 μl of cDNA in a total reaction volume of 50 μl. The template cDNA was denatured for 5 min at 94°C, after which 35 amplification cycles (94°C for 20 s, 50°C for 20 s, and 72°C for 1 min) were carried out (GeneAmp PCR System 9700, Applied Biosystems, Foster City, CA). The PCR products (5 μl per sample) and size standards were electrophoresed in a 1.5% agarose gel and stained with ethidium bromide. Samples were scored as plus versus minus based on presence of band.

Viral loads or titer per bee was measured using real-time quantitative PCR, by using the Brilliant SYBR Green QPCR core reagent kit (Stratagene, Stratagene, La Jolla, CA). Specific primers were designed with PRIMER EXPRESS software (Applied Biosystems) for DWV and β-actin as follows: DWV (5′-GACAAAATGACGAGGAGATTGTT-3′ and 5′-CAACTACCTGTAATGTCGTCGTGTT-3′) and β-actin (5′-ATGCCAACACTGTCCTTTCTGG-3′ and 5′-GACCCACCAATCCATACGGA-3′). The PCR reaction contained 1X core PCR buffer, 2 mM MgCl$_2$, 0.8 mM dNTP mix, 8% glycerol, 3% dimethyl sulfoxide, 300 nM reference dye, 0.5X SYBR Green I dye (Stratagene), 0.05 U/μl SureStart *Taq*DNA polymerase (Stratagene), 100 nM both forward and reverse primers, and an appropriate volume of Milli-Q water (Mil-

lipore Corporation, Billerica, MA) to bring the total volume to 25 μl. The PCR measurements were duplicated on two separate plates. The PCR reactions were carried out in an ABI PRISM 7700 sequence detection system (with SDS 1.9.1.software package, Applied Biosystems) by using the following settings: one cycle of a 10-min preincubation at 95°C, and 40 cycles of amplification with 30 s of denaturing at 95°C, 1 min of annealing at 59°C, and a 1-min extension at 72°C. Relative quantifications were calculated by using the comparative C_T method against the expression levels of β-actin in the respective samples. The expression levels of β-actin were highly similar in different groups of bees and treatments. Melting curves, DNA agarose gels, and DNA sequencing were used to confirm the specificity of PCR amplifications.

Statistical Methods. The effects of treatment on the mean abundance of varroa mites, average daily varroa mite drop, mite viability in brood cells, amount of brood, and DWV prevalence and load were analyzed using a Before-After Control-Impact (BACI) design (Stewart-Oaten et al. 1986, Smith 2002). A repeated measures analysis of variance (ANOVA) was performed using colonies as replicates and an unstructured covariance structure (PROC MIXED, SAS Institute 1999). Varroa mite mean abundance on adult bees, prevalence in brood cells, proportion of dead mites in brood, and DWV prevalence were arcsine transformed, whereas average daily varroa mite drop, frames of drone and worker brood, and intensity of mite infestation in brood were square-root transformed before analysis. When the apiary was found to have a significant effect ($P < 0.05$), data from apiaries were analyzed separately. Otherwise, data from all apiaries were pooled. When significant interactions between treatment and time (before and after) were found ($P < 0.05$), contrasts were used to compare changes in all factors over time in water-treated (control) colonies to the changes in the other treatments over time by using Bonferroni-corrected α values. The effect of treatment on varroa mite drop during the 17 h of fumigation was analyzed using ANOVA (PROC GLM, SAS Institute 1999) with treatment and apiary as the main effects. When a significant treatment × apiary effect was found, the effect of treatment within each apiary was examined separately.

In the subset of colonies for which examined cells had their position on the frame and/or the frame's position in the colony recorded, the proportion of dead mites found on different frames, or in different places on the same frame, were compared using the Wilcoxon paired sample test (NPAR1WAY, SAS Institute 1999). Fisher exact test was used to determine whether treatment increased the incidence of queenlessness (PROC FREQ, SAS Institute 1999).

Results

Impact of Treatment on Average Daily Mite Drop. Average daily mite drop onto sticky boards was affected by treatment over time ($F = 2.95$; df $= 3, 47$; $P = 0.0422$; Fig. 1). The change in average daily mite drop from
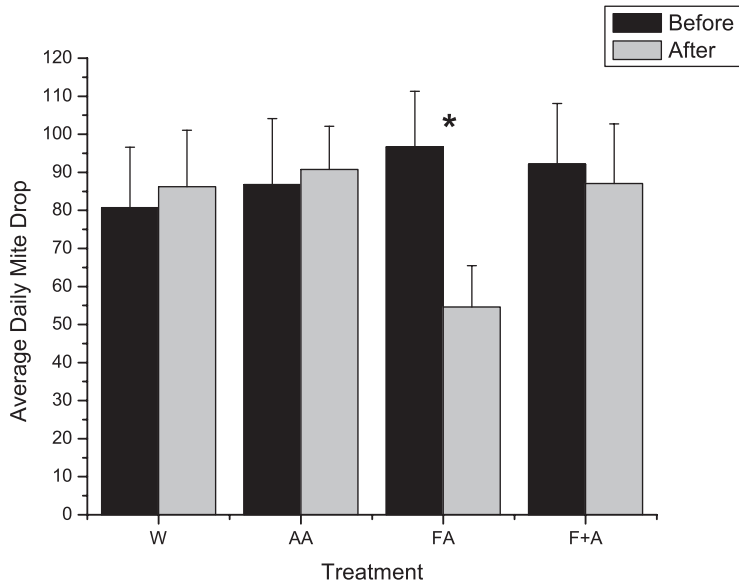
Fig. 1. Average daily mite drop (mean ± SE) before and 4 wk after a 17-h treatment with a water control (W), acetic acid (AA), formic acid (FA), or both acetic and formic acids (F + A).

before treatment to 4 wk after treatment was not affected by apiary, as indicated by a nonsignificant apiary × treatment × time interaction ($F = 0.26$; df $= 5, 40$; $P = 0.9302$). The average daily mite drop decreased over the 4-wk study period in FA-treated colonies compared with the increase observed in the water-treated control colonies ($F = 6.60$; df $= 1, 47$; $P = 0.0405$). The change in average daily mite drop in AA- and F + A-treated colonies did not differ from the change observed in the water control group ($F = 0.01$; df $= 1, 47$; $P = 1.00$ and $F = 0.29$; df $= 1, 47$; $P = 1.00$, respectively).

Impact of Treatment on Average Mite Drop during the 17-h Fumigation Period. The number of varroa mites that dropped onto sticky boards during the treatment period was affected by apiary as indicated by a significant treatment × apiary effect ($F = 3.91$; df $= 5, 40$; $P = 0.0056$). Therefore, treatment effect was determined separately in each apiary. In the Way Har apiary, treatment significantly affected mite drop ($F = 13.45$; df $= 3, 16$; $P = 0.0001$; Fig. 2) with the FA and F + A treatments causing more mite drop than both the AA and W treatments. In the Grange apiary, treat-
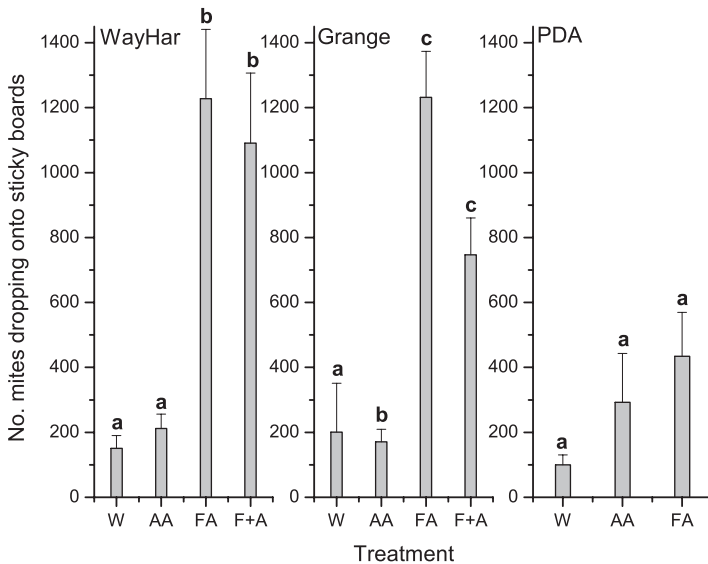

Fig. 2. Average varroa mite drop (mean ± SE) onto sticky boards during a 17-h treatment application with a water control (W), acetic acid (AA), formic acid (FA), or both acetic and formic acids (F + A) in three separate apiaries. Bars with the same letter are not significantly different from each other ($P > 0.05$) within each apiary.
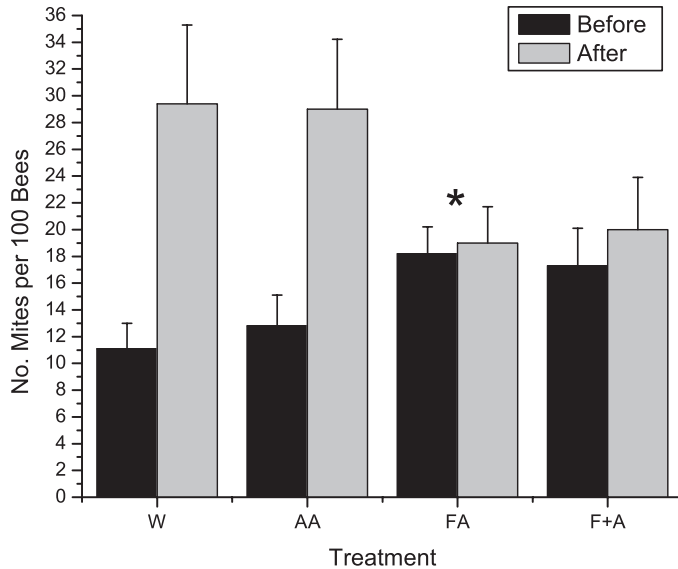
Fig. 3. Mean abundance of varroa mites (mean $\pm$ SE) before and 4 wk after a 17-h treatment with a water control (W), acetic acid (AA), formic acid (FA), or both acetic and formic acids (F + A). An asterisk indicates a significant change in mean abundance of varroa mites in treated colonies compared with the change in mean abundance in the water control colonies ($P < 0.05$).

ment significantly affected mite drop during the 17-h treatment period ($F = 26.08$; df $= 3, 15$; $P < 0.0001$), with the FA treatment causing significantly more mites to drop in the 17-h treatment period than any other treatment, and the F + A treatment causing more than the AA or W treatments (Fig. 2). In contrast, treatment did not affect the change in mite drop onto sticky boards in the PDA apiary ($F = 2.00$; df $= 2, 11$; $P = 0.1908$).

Impact of Treatment on Mean Abundance of Varroa Mites. The change in mean abundance of varroa mites (mites per 100 bees) over the 4 wk of study was affected by treatment ($F = 3.35$; df $= 3, 47$; $P = 0.0266$; Fig. 3). Apiary did not have an impact as indicated by a nonsignificant apiary $\times$ treatment $\times$ time interaction ($F = 0.70$; df $= 5, 40$; $P = 0.6291$). Over the course of this study, mean abundance of varroa mites increased in all treatment groups. Only the FA-treated colonies had a significantly smaller increase than the water-treated control group ($F = 6.52$; df $= 1, 47$; $P = 0.0417$). The change in mean abundance of mites in the AA- and F + A-treated colonies were not significantly different than the change observed in water-treated colonies ($F = 0.09$; df $= 1, 47$; $P = 1.00$ and $F = 4.42$; df $= 1, 47$; $P = 0.1443$, respectively).

Impact of Treatment on Varroa Mites in Brood. Varroa mite prevalence (before, $19.4 \pm 1.6\%$ versus after, $24.1 \pm 2.2\%$ of cells infested) and intensity (before, 1.3 ± 0.03 versus after, 1.3 ± 0.04 mites per infested cell) in worker brood were not affected by treatment over time ($F = 1.72$; df $= 3, 46$; $P = 0.7653$ and $F = 2.38$; df $= 3, 46$; $P = 0.0821$, respectively). However, the proportion of dead mites in worker brood cells was significantly impacted by treatment ($F = 21.64$; df $= 3, 46$; $P < 0.0001$; Fig. 4) but not by

apiary, as indicated by a nonsignificant apiary $\times$ treatment $\times$ time interaction ($F = 0.84$; df $= 5, 39$; $P = 0.5281$). Both the FA- and F + A-treated colonies had significantly higher mite mortality in worker brood cells ($F = 45.27$; df $= 1, 46$; $P < 0.0003$ and $F = 18.67$; df $= 1, 46$; $P < 0.0003$, respectively), compared with the water control treatments or the AA treatment ($F = 0.24$; df $= 1, 46$; $P = 1.00$).

The distribution of the dead mites in the brood frames varied between the upper versus lower brood chambers in the FA-treated colonies. In the lower brood chamber, the distribution of dead mites in brood cells was not different across the central frame, as the proportion of dead mites found in the upper half was the same as that in the lower half of the brood frames ($Z = 1.62$, $P = 0.10$; top, 0.696 ± 0.0596, $n = 51$ colonies; bottom, 0.548 ± 0.0640, $n = 55$ colonies). Given this lack of difference, the data on mite mortality in brood cells in the central frame in the bottom chamber were pooled, and the proportion of dead mites on those frames was compared with the proportion of dead mites found in frames on the periphery of the bottom brood chamber or on central frames in the upper brood chamber. Differences in the proportion of dead mites in worker brood originating from different frames in the brood nest were detected (Fig. 5), with the greatest proportion of dead mites found in frames in the upper brood chamber.

Prevalence (before, $58.4 \pm 3.3\%$ versus after, $62.1 \pm 6.7\%$ of cells infested) and intensity of mite infestation (before, 2.4 ± 0.1 versus after, 2.7 ± 0.2 mites per infested cell) and the proportion of dead mites (before, 0.16 ± 0.04 versus after, 0.01 ± 0.03) in drone brood were not affected by treatment over time ($P > 0.05$).
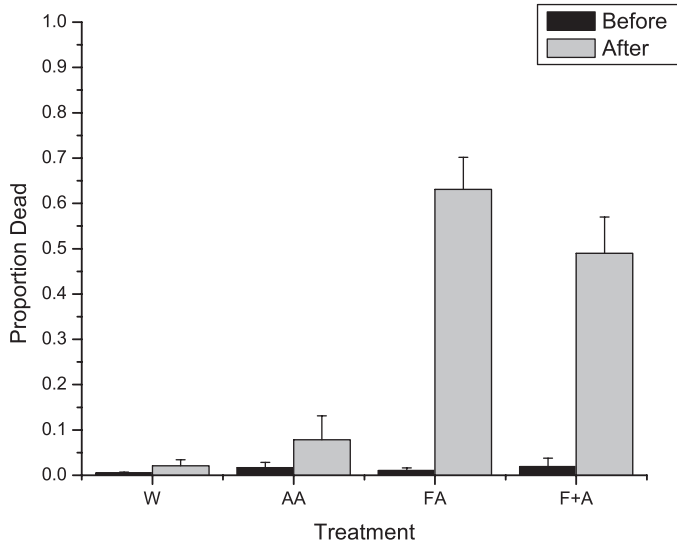
Fig. 4.   Proportion of mites found dead (mean ± SE, dead mites/total mites) in worker brood before and after a 17-h treatment with a water control (W), acetic acid (AA), formic acid (FA), or both acetic and formic acids (F + A).

Impact of Treatment on Brood. The number of frames of drone brood found in colonies was not affected by treatment over time (before, 0.129 ± 0.019 versus after, 0.108 ± 0.018 frames; $n = 51$ colonies; $F = 2.53$; df = 3, 47; $P = 0.0686$). The change in the number of frames of worker brood found in colonies also was not affected by treatment over time (before, 3.27 ± 0.39 versus after, 1.64 ± 0.173 frames; $n = 51$; $F = 0.60$; df = 3, 47; $P = 0.6203$).

Impact of Treatment on Queens. Ten of the 51 colonies included in this study lost queens over the period of study. However, the mite treatments themselves did not affect queen survival ($P = 0.3905$; Fisher exact test).
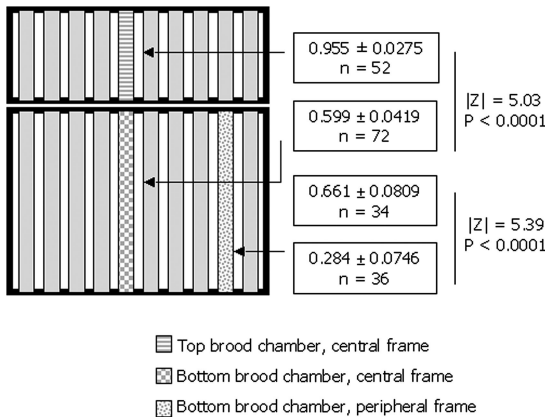

Fig. 5.   View of a transected colony, revealing frames in the upper and lower brood chambers. Frames examined in formic acid-treated colonies ($n = 5$) are highlighted and differences in the proportion of dead mites in worker brood (LSMean ± SE) originating from different frames are presented (Wilcoxon two-sample test).

Impact of Treatment on DWV. The change in DWV prevalence (before, 0.83 ± 0.01% versus after, 0.82 ± 0.08% of bees infected) did not differ among treatment groups over time ($F = 2.56$; df = 2, 7; $P = 0.1468$). Viral load was highly variable among bees before treatment (19,088 ± 1,722 normalized DWV virus mRNA level per actin RNA level). One bee had a viral load 8.6 times the standard deviation of the viral load; thus, it was removed from the analysis. Mean viral load in colonies decreased in colonies over time (from 7,393 ± 2,365 to 1,908 ± 542 normalized DWV level; $n = 9$; $F = 8.31$, df = 1, 8; $P = 0.024$; Fig. 6). However, this decrease was the same among the different treatment groups as indicated by a nonsignificant treatment × time interaction ($F = 2.28$, df = 2, 6; $P = 0.1355$).

Temperature during Treatment. During treatment, the temperature averaged 22.0 ± 2.4°C (range 17.7–27.2°C) in the Grange apiary, 22.7 ± 1.2°C (range 17.2–28.8°C) in the PDA apiary, and 23.6 ± 1.1°C (range 21.1–27.2°C) in the Way Har apiary.

Discussion

The 17-h formic acid treatment tested in this study was an effective varroa mite control technique. Most notably, under relatively hot ambient temperatures during a late-season nectar flow, the 50% formic acid treatment killed a significant proportion of varroa mites in worker brood without increasing queen or brood mortality. The treatment effect on the varroa mite population was detected using several different methods to estimate mite populations. This type of formic acid treatment prevented a significant increase in the mean abundance of varroa mites on adult bees in treated colonies and lowered average daily mite drop four weeks after treatment. In contrast, acetic
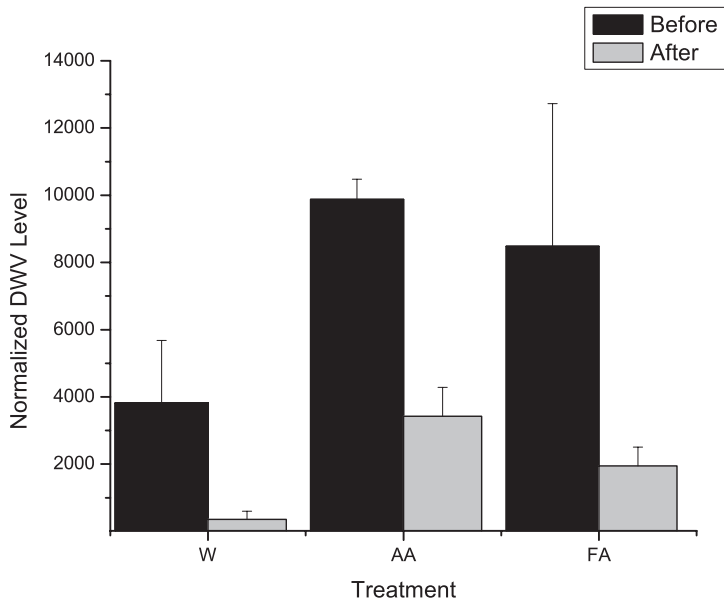
Fig. 6. Mean ± SE viral load before and after a 17-h treatment with a water control (W), acetic acid (AA), or formic acid (FA).

acid was not effective as a mite control agent and the addition of acetic acid to the formic acid treatment decreased treatment efficacy. Short-term acid fumigation had no effect on DWV prevalence or viral load.

Varroa Mites in Brood Cells. The 50% formic acid treatment for 17 h killed >60% of the mites in brood cells. This degree of mite mortality under cappings was similar to that reported in a study by Calderon et al. (2000), in which treatment killed 55.6% of adult varroa mites in sealed brood of Africanized honey bees in Costa Rica. Fries (1991) also reported mites being killed in brood during formic acid treatment but removed the adult bees from the brood frames for treatment, a time-consuming method unlikely to be accepted by commercial beekeepers.

Mites in capped worker brood close to the site of formic acid application were more likely to be killed than those farther away, which was also reported by Fries (1991). Thus, the location of drone brood on the periphery of the frames at a site somewhat removed from the application may explain why treatment did not kill mites infesting drone brood.

The impact of treatment on mite populations underneath cappings was significantly reduced when acetic acid was added to the fumigation boards. Formic acid resulted in average mite mortality of 63% below the brood cappings, whereas mite mortality in brood cells was 49% for formic and acetic acid-treated colonies. This small difference in mite mortality beneath brood cappings had a significant, pronounced effect on mite populations at 4 wk after treatment, as measured by average daily mite drop and mean abundance (Figs. 1 and 3). This finding is not surprising, considering that a majority of mites is beneath brood cappings when colonies are actively rearing brood. This finding also suggests that efforts to develop short-

term mite control treatments should focus on maximizing mite mortality in brood cells.

Varroa Mites on Adult Bees. By monitoring average daily mite drop, we found that the varroa mite population decreased significantly in colonies treated with formic acid alone (43% reduction), decreased slightly in colonies treated with both formic and acetic acids (8% reduction), and increased in the acetic acid-treated colonies at the same rate as observed in water-treated control colonies (5 and 6% increase, respectively). Similar results were found when comparing mean abundance of varroa mites on adult bees, another measure of the varroa mite population in colonies. The number of mites per 100 adult bees increased dramatically in the acetic acid- and water-treated colonies (123 and 150% increase, respectively), whereas increasing only slightly in the colonies treated with formic acid or a combination of formic and acetic acids (5 and 18% increase, respectively). These results, with treatment lasting only 17 h, are similar to those reported by others treating for 21 d with 250 ml of 65% formic acid (Calderone 1999, Calderone and Nasr 1999, Elzen et al. 2004).

When formic and acetic acids were used simultaneously to treat colonies, the effect of treatment on mites dropping during the treatment period was similar to the mite drop induced by formic acid alone, with both treatments removing large numbers of mites. Acetic acid alone was not an effective mite control treatment. Treatment did not affect daily mite drop, mite drop during the 17-h fumigation, mean abundance of varroa mites, or the proportion of dead mites in brood cells.

Deformed Wing Virus. Even though the mite populations were reduced, the prevalence of DWV in colonies did not change over the 4-wk study period.

Although viral load did decrease over the same period, the change was not affected by treatment. The decrease in viral load recorded here can be explained considering the population structure of the experimental colonies. When the first samples were removed from colonies, colonies were actively rearing brood, and because samples were removed from the brood nest, it is reasonable to assume that the bees collected were young. DWV is known to shorten the longevity of infected individuals (Yang and Cox-Foster 2005, 2007). Therefore, it is likely that those individuals with high viral loads would not have survived in the colony beyond several days. During the second collection period in late summer, brood production had been reduced and many of the bees collected in the brood nest were apt to be older compared with the age of bees collected from the brood nest when brood production was high. If bees with high viral loads die sooner than less-infected individuals, it is logical to expect bees with higher average ages to have lower viral loads. However, this hypothesis requires in-field verification.

Our failure to demonstrate a reduction in virus prevalence is troubling in that it demonstrates that removal of mites in late summer does not have an immediate effect on virus levels in colonies and the viral infections in these colonies is at high levels entering winter when most varroa/virus colony loss occurs. The bees sampled in this study were likely representative of the colonies' winter bees. The levels of virus found in this study were higher than those reported by Yang and Cox-Foster (2005) from bees emerging with deformed wings. Increased levels of virus due to varroa mite parasitism decrease the life span of honey bees (Yang and Cox-Foster 2007), thus contributing to colony death and making the need to control viruses paramount. Our findings in this study suggest that early or mid-season control of varroa mites may be necessary to prevent the amplification of DWV in colonies and to prevent the adverse effects of DWV and mites on colony survival.

In summary, although the level of mite mortality measured in this study was insufficient for a stand-alone treatment (Calis et al. 1998), 50% formic acid treatment for 17 h does hold considerable promise as a tool for beekeepers that need to control mites during the nectar flow. This short-term fumigation method is highly advantageous compared with previously used, long-term methods that had to remain in place for 4 wk to ensure that mites emerging from brood were treated (Bracey and Fischer 1989). Additionally, prolonged treatment negatively affects honey bee brood care and feeding (Bolli et al. 1993). In this study, the short-term treatment with 50% formic acid for 17 h did not negatively impact brood production or queen survivorship.

A treatment that takes <24 h and kills >50% of varroa mites in brood may be a useful tool for beekeepers that need to treat for varroa mites during the nectar flow. The short-term method tested in this study meets these criteria; thus, it holds considerable promise as a tool beekeepers can incorporate into their mite control arsenal.

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