THIS PAPER PROVES THAT A SLOW - LOW DOSE - PROLONGED TREATMENT WITH FORMIC ACID WILL PROVIDE SUFFICIENT CONTROL OF VARROA.

Effect of Concentration and Exposure Time on Treatment Efficacy Against Varroa Mites (Acari: Varroidae) During Indoor Winter Fumigation of Honey Bees (Hymenoptera: Apidae) with Formic Acid

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ABSTRACT The combination of the concentration of formic acid and the duration of fumigation (CT product) during indoor treatments of honey bee, *Apis mellifera* L., colonies to control the varroa mite, *Varroa destructor* Anderson & Trueman, determines the efficacy of the treatment. Because high concentrations can cause queen mortality, we hypothesized that a high CT product given as a low concentration over a long exposure time rather than as a high concentration over a short exposure time would allow effective control of varroa mites without the detrimental effects on queens. The objective of this study was to assess different combinations of formic acid concentration and exposure time with similar CT products in controlling varroa mites while minimizing the effect on worker and queen honey bees. Treated colonies were exposed to a low, medium, or high concentration of formic acid until a mean CT product of 471 ppm*d in room air was realized. The treatments consisted of a long-term low concentration of 19 ppm for 27 d, a medium-term medium concentration of 42 ppm for 10 d, a short-term high concentration of 53 ppm for 9 d, and an untreated control. Both short-term high-concentration and medium-term medium-concentration fumigation with formic acid killed varroa mites, with averages of 93 and 83% mortality, respectively, but both treatments also were associated with an increase in mortality of worker bees, queen bees, or both. Long-term low-concentration fumigation had lower efficacy (60% varroa mite mortality), but it did not increase worker or queen bee mortality. This trend differed slightly in colonies from two different beekeepers. Varroa mite mean abundance was significantly decreased in all three acid treatments relative to the control. Daily worker mortality was significantly increased by the short-term high concentration treatment, which was reflected by a decrease in the size of the worker population, but not an increase in colony mortality. Queen mortality was significantly greater under the medium-term medium concentration and the short-term high concentration treatments than in controls.

KEY WORDS treatment, *Apis mellifera*, *Varroa destructor*, CT product

FUMIGATING HONEY BEE, *Apis mellifera* L., colonies with formic acid is a feasible option to control varroa mites, *Varroa destructor* Anderson & Trueman, and other pests. Formic acid can be applied in the field during the spring or fall (Krämer 1986, Bracey and Fischer 1989, Feldlaufer et al. 1997, Daniels et al. 1999, Kochansky and Shimanuki 1999, Calderone 2000, Skinner et al. 2001, Gatien and Currie 2003, Ostermann and Currie 2004) or indoors during the winter (Underwood and Currie 2004). Indoor fumigation looks promising and has the advantages of control over ambient temperature and added susceptibility of mites because of a lack of honey bee brood (Underwood and Currie 2003, 2004).

Standardization of fumigation methods for reliable pest control requires the determination of the most effective combination of the formic acid concentration and exposure time, which must be studied under the range of environmental conditions that may be present during use of the fumigant (Harein and Krause 1964, Estes 1965, Monro 1969, Underwood and Currie 2003). Generally, as the concentration of the fumigant increases, the amount of time necessary for effective pest control decreases and vice versa (Harein and Krause 1964). The combination of concentration and exposure time can be expressed as a value known as the concentration × time (CT) product (Monro 1969). For some species, this value remains relatively constant over a range of exposure times, allowing it to be used in a variety of practical situations (Monro 1969).

In the laboratory, Underwood and Currie (2003) determined the CT_{50} product for varroa mites at various temperatures and formic acid concentrations. This value was then used as a basis for tests of entire colonies in a wintering building (Underwood and Currie 2004). A CT product of 37 ppm*d in hive air (82 ppm*d in room air) given over 2 d is effective at killing varroa mites, but it also causes significant queen loss (Underwood and Currie 2004) and a CT product of 70

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ppm*d (114 ppm*d in room air) given over 5 d had a similar effect (Underwood 2005). Queen loss during formic acid fumigation in the wintering building is thought to be associated with the peak formic acid measurement in the hive rather than with the CT product to which the queens are exposed (Underwood 2005). Therefore, we hypothesized that if the colonies are exposed to a relatively high CT product given as a low concentration of formic acid over a long exposure time, it might prevent high peak concentrations of formic acid in hives and provide good efficacy without queen loss. The objective of this study was to assess varying combinations of formic acid concentration and exposure time with similar CT products to determine the efficacy of each in controlling varroa mites while minimizing the effect of treatment on worker and queen honey bees.

Materials and Methods

Before Experimentation. Eighty-four single-chamber Langstroth hives were obtained from a commercial beekeeper (AF; 32 hives) or the University of Manitoba (UM; 52 hives) in late fall 2001. Pretreatment samples were collected before the colonies were moved indoors to estimate the mean abundance of varroa mites and the size of the honey bee population. Initial mean abundance of varroa mites was measured from a sample of 200–300 workers from each colony by using an alcohol wash method with a mechanical shaker (Gatien and Currie 2003). Voucher specimens of mites from each beekeeper are held in the J. B. Wallis Museum of Entomology (Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada). The honey bee population size was estimated by counting the number of frames completely covered with bees and multiplying by 2,430 bees (Burgett and Burikam 1985).

On 11 December 2001, colonies were weighed and brought indoors where they were positioned in stacks placed randomly in a large holding room (5.2 by 7.2 by 2.5 m) before the start of the experiment. Each hive had a completely open bottom entrance (37 by 2.5 cm) and a small top entrance (AF, 1.9 by 0.7 cm; UM, 5.7 by 1.9 cm). On 28 December 2001, colonies were sorted by beekeeper and initial mean abundance of varroa mites and divided into 21 blocks of four hives each. One hive from each block was randomly assigned to a position within each treatment room. Hives were placed in five adjacent columns against one wall, with the middle column containing a stack of five hives and the others containing stacks of four hives.

Hives were fitted with a piece of white poster board the size of the bottom board of the hive. This board was placed on the bottom board of each hive to collect worker and queen bees and varroa mites falling from the honey bee cluster. White board contents were collected for a 7-d pretreatment period and daily for 36 d during and immediately after fumigation. On 15 February, stacks of hives were again placed randomly in the holding room where white board contents were collected biweekly until colonies were moved outdoors.

Fumigation. Fumigation tests were conducted in four 3.0 by 1.7 by 2.5-m treatment rooms in the wintering building at the University of Manitoba (Winnipeg, Manitoba, Canada) (N 49° 48′ 32″, W 97° 07′ 37″). Each room simulated the airflow conditions of a standard commercial wintering building with a fan jet air distribution system (Gruszka 1998), but flow was modified to allow formic acid fumigation (see Underwood and Currie 2004 for details and schematic diagrams). To distribute the formic acid throughout the treatment rooms, a fan and pan system was set up in an air mixing chamber according to the methods of Underwood and Currie (2004). A pan (25.0 by 25.0 by 5.5 cm) of liquid was placed in front of a fan (10-cm-diameter window fan, Holmes Products Corp., Milford, MA). A 10-liter plastic bag (Reliance Products Ltd., Winnipeg, Manitoba, Canada) was connected to the pan by a tube (0.4-cm inner diameter, Anderson Barrows Metals Corp., Palmdale, CA). The bag acted as a reservoir to replace liquid that evaporated from the pan. Flow of liquid between the bag and the pan was regulated by a float system (Air-King Ltd., Brampton, Ontario, Canada). Ventilation during fumigation was 0.4 liters/s/m^3. Before and after fumigation, temperature and ventilation in all rooms was controlled with three additional stepwise fans set on thermostats at 1°C increments that switched on in series when room temperature rose above 6°C (Gruszka 1998).

During the experiment, one room, the control, remained untreated, with no formic acid exposure. The other three rooms were fumigated with formic acid by using different treatment regimes. Three application methods were designed to expose honey bee colonies in different treatment rooms to different concentrations of formic acid. The pan in the long-term low-concentration treatment started with 1 liter of 25% formic acid, a humidifier sponge (30 by 19 by 5 cm, Air-King Ltd.), and a reservoir bag with 3 liters of 65% formic acid. The pan in the medium-term medium-concentration treatment started with 1 liter of 40% formic acid, three T-shaped humidifier plates (11.5 by 16.7 by 0.2 cm, 64–3119-0, Dundas-Jafine Industries Ltd., Weston, Ontario, Canada), and a reservoir bag with 3 liters of 65% formic acid. The pan in the short-term high-concentration treatment started with 1 liter of 65% formic acid, three T-shaped humidifer plates, and a reservoir bag with 3 liters of 85% formic acid. Reservoir bags were refilled as required throughout the fumigation period. The total amount of liquid evaporated during fumigation was 6.7 liters in the long-term low-concentration treatment room, 8.0 liters in the medium-term medium-concentration treatment room, and 7.6 liters in the short-term high-concentration treatment room.

Starting on 11 January, treated colonies were exposed to a low, medium, or high concentration of formic acid until a mean CT product of ≈471 ppm*d (based on room air) was realized. This value was determined by allowing the short-term high-concentration treatment to continue until substantial worker

mortality was observed. The CT product from the previous day, when worker mortality was not yet observed, was 482 ppm*d in room air. This short-term high-concentration treatment consisted of an average room air concentration of 52.5 ± 3.6 ppm formic acid for 9 d (worker mortality was evident on the tenth day). The other two formic acid fumigated rooms then continued until they reached a similar CT product. The medium-term medium-concentration treatment was 42.2 ± 3.3 ppm formic acid for 10 d (439 ppm*d) and the long-term low-concentration treatment was 18.9 ± 1.1 ppm formic acid for 27 d (492 ppm*d). The CT product for each room was calculated by summing the mean daily room air concentrations during fumigation.

Formic acid concentration in treatment room and hive air was measured using either a PortaSens II gas leak detector (00-1038 Acids Sensor, Analytical Technology, Inc., Oaks, PA) or by using Dräger tubes (Tube No. 6722101 with Accuro 9022003 gas detector pump, National Dräger, Pittsburgh, PA). The PortaSens II was calibrated against Dräger tubes by using a standard curve so that each device would provide equivalent results. Samples were collected from polyethylene air-sampling tubes (4.3-mm inner diameter, Anderson Barrows Metals Corp.) that ran from the point of measurement to a site outside the treatment room where they were sealed with rubber sleeve stoppers (5.2–6.7-mm plug diameter, 03–215-5, Fisher, Ottawa, Ontario, Canada) according to the methods of Underwood and Currie (2004). Measurements were taken from within hives at a point near the center of the honey bee cluster of the bottom, middle, and top hives in the center, five-hive stack and from the center of each acid-fumigated treatment room at heights of 0.1, 0.5, and 1.5 m above the floor. Sampling tubes were inserted into the hives through a 6-mm-diameter hole in the center of the face of the hive box. Formic acid concentration in the rooms and in the three hives within each room was sampled daily during fumigation and after fumigation until the concentration dropped below one ppm. A HOBO data logger (Onset Computer Corp., Bourne, MA) was placed in the center of each treatment room 1.5 m above the floor near the opening of the air sampling tube, where temperature and humidity were measured every 30 min throughout the experiment starting 1 wk before fumigation.

To collect white board samples of varroa mites and bees during fumigation, a low pressure ambient air breathing apparatus (model 1023-P152G-G608X, Gast Manufacturing Corp., Benton Harbor, MI) with continuous flow supplied air hood (model R799, Wilson Safety Products, Reading, PA) was used. This allowed the experimenter to enter the experimental rooms safely during fumigation at the concentrations of formic acid used in our experiments.

After Fumigation. The bees were moved outside on 8 April 2002 when all colonies were assessed to determine hive weight, honey bee population size, and varroa mite mean abundance, as described above. The total number of mites that were present during fumigation was derived by estimating the total number of mites remaining in the colonies when they were moved outdoors, a time when little or no brood was present in the hives. The mean abundance of varroa mites from the 8 April alcohol wash samples was multiplied by the size of the honey bee population. This number was then added to the total number of mites collected in white board samples during and after fumigation to estimate the total number of mites present at the start of fumigation. Proportional mite mortality values were then obtained by dividing the cumulative daily mite count to day i by the total number of mites at the start of fumigation.

On 17 April 2002, the state of the colony (queenright or queenless) was assessed by the observation of eggs or the queen and colony survival was determined. On 10 May 2002, the size of the honey bee brood population was estimated. The brood area (square centimeters), of both capped and uncapped brood, was estimated using a piece of Plexiglas the size of a frame marked with a 1-cm grid.

Statistical Analysis. Split-plot analysis of variance (ANOVA) tests were used to analyze formic acid concentration data. Separate analyses were done on air from treatment rooms and hives (PROC GLM, SAS Institute 1999). Treatment room and sample height were the main plot factors, and exposure time was the subplot factor. Treatment room × sample height was the error term for the main plot factors. When significant treatment room differences were found ($P < 0.05$), a Student–Newman–Keuls multiple range test was used to compare differences between means (SAS Institute 1999).

A split-plot ANOVA test was also used to analyze cumulative varroa mite and worker bee mortality and change in hive weight. Varroa mite mortality data were weighted by the total number of mites present in each colony at the start of fumigation because the mite populations were unequal (Snedecor and Cochran 1980). The main plot factors were treatment room, beekeeper (AF or UM), and replicate and the subplot factor was exposure time. Treatment room × beekeeper × replicate was used as the error term for the main plot factors and the interactions between them. When a significant three-way interaction between treatment room, beekeeper and exposure time was found, separate ANOVA tests were run on varroa mite and worker bee mortality data from each beekeeper's colonies. Differences between treatments on the final sampling day were analyzed using Duncan's multiple range test (SAS Institute 1999).

The effect of treatment on mean abundance of varroa mites and the worker bee population size was analyzed as a before-after control-impact (BACI) design (Stewart-Oaten et al. 1986, Smith 2002) where hives were treated as replicates by using a repeated measures ANOVA with a heterogeneous autoregressive covariance structure (PROC MIXED, SAS Institute 1999). The main plot factors were treatment room and beekeeper (AF or UM). Mean abundance of varroa mite data were arcsine transformed, whereas worker bee population estimates were log transformed before analysis. There was no treatment

room × beekeeper × exposure time interaction (F = 0.88; df = 3, 62; $P > 0.05$), so the data from the two beekeepers was pooled. When significant interactions between treatment room and exposure time were found ($P < 0.05$) contrasts were used to compare changes of each factor over time in each treatment room to changes over time in the control using Bonferroni-corrected α values.

The effect of treatment room on queen bee and colony survival was analyzed by using Fisher's exact test and comparing each treatment to the control (SAS Institute 1999). The effect of treatment room on pretreatment varroa mite and worker bee drop, and on the area of capped brood, uncapped brood, and total brood area on 10 May was analyzed as a completely randomized design by using an ANOVA. The influence of beekeeper on the initial weight and cluster size of colonies was analyzed as a completely randomized design by using an ANOVA.

Results

Formic Acid Concentration. When all three acid-treated rooms were being fumigated simultaneously (during the first 9 d of fumigation), there was no effect of stacking height (F = 0.62; df = 2, 4; $P > 0.05$) or treatment room on formic acid concentration in hive air over time (F = 1.14; df = 16, 32; $P > 0.05$). There also was no effect of sample height on the concentration of formic acid in room air (F = 3.57; df = 2, 4; $P > 0.05$), but, as planned, all rooms had different formic acid concentrations (F = 76.71; df = 2, 27; $P < 0.001$; Student–Newman–Keuls; Fig. 1). During the 27 d of fumigation in the long-term low-concentration treatment room, formic acid concentration in room air averaged 18.9 ± 1.1 ppm (range 2.8–40.7 ppm) and in hive air averaged 6.0 ± 0.4 ppm (range 2.7–17.6 ppm). During the 10 d of fumigation in the medium-term medium-concentration treatment room, formic acid concentration in room air averaged 42.2 ± 3.3 ppm (range 5.6–67.7 ppm) and in hive air averaged 15.5 ± 3.4 ppm (range 2.7–59.7 ppm). During the 9 d of fumigation in the short-term high concentration treatment room, formic acid concentration in room air averaged 52.5 ± 3.6 ppm (range 3.3–68.6 ppm) and in hive air averaged 12.3 ± 3.1 ppm (range 2.8–59.4 ppm).

Varroa Mite Daily Mortality. Before fumigation, there was no effect of treatment room on varroa mite mortality (F = 1.89; df = 2, 40; $P > 0.05$). During fumigation, there was a significant treatment room × beekeeper × exposure time interaction for cumulative varroa mite mortality (F = 2.02; df = 123, 534; $P < 0.0001$). Formic acid treatment caused significant mite mortality in colonies from both beekeepers (AF and UM) as indicated by significant treatment room × exposure time interactions (AF: F = 10.31; df = 123, 780; $P < 0.0001$; UM: F = 20.71; df = 123, 984; $P < 0.0001$; Fig. 2). However, the response to different concentrations varied between beekeepers. Varroa mites tended to be killed at a faster rate and had a greater rate of overall mortality in the colonies from UM than in the colonies from AF (Fig. 2). The final mite mortality rate in UM colonies was 21.5 ± 4.5% (n = 11) in the control, 81.6 ± 3.7% (n = 12) in the long-term low-concentration treatment, 100 ± 3.6% (n = 10) in the medium-term medium-concentration treatment, and 100 ± 5.5% (n = 7) in the short-term high-concentration treatment (Fig. 2). The final mite mortality rate in AF colonies was 7.5 ± 10.2% (n = 8) in the control, 42.8 ± 11.0% (n = 8) in the long-term low-concentration treatment, 66.0 ± 11.0% (n = 8) in the medium-term medium-concentration treatment, and 84.5 ± 11.9% (n = 6) in the short-term high-concentration treatment (Fig. 2).

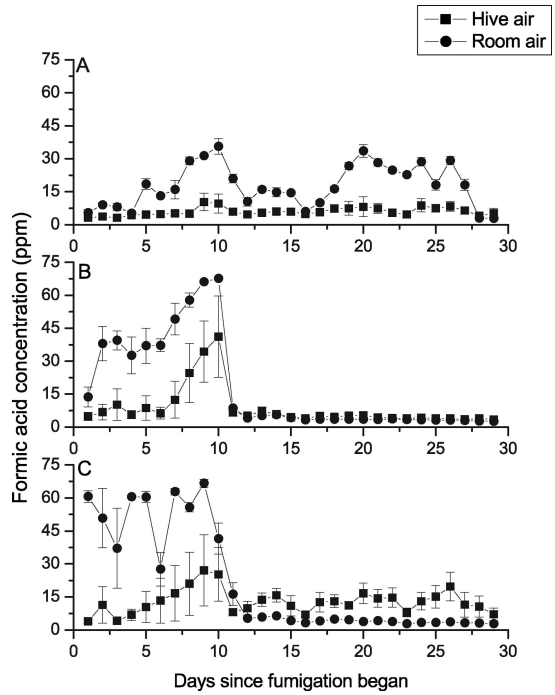

Fig. 1. Concentration of formic acid (mean ± SE) in room and hive air during fumigation in rooms receiving a low concentration for 27 d (A), a medium concentration for 10 d (B), or a high concentration for 9 d (C). n = 3 for each point.

Worker Bee Daily Mortality. Before fumigation, there was no effect of treatment room on worker bee mortality (F = 3.05; df = 2, 40; $P > 0.05$). There was no significant treatment room × beekeeper × exposure time interaction for cumulative worker bee mortality (F = 0.17; df = 123, 534; $P > 0.05$); however, there was a significant treatment room × exposure time interaction (F = 11.37; df = 123, 534; $P < 0.0001$; Fig. 3). Bee mortality was not significantly different between treatment rooms until day 10, when bee mortality in the short-term high-concentration treatment was significantly higher than the control and in all other treatments (Fig. 3). From day 11 until day 83, bee mortality rates in all treatment rooms were fairly consistent (Fig. 3). There was no treatment room × beekeeper effect (F = 0.92; df = 3, 12; $P > 0.05$).

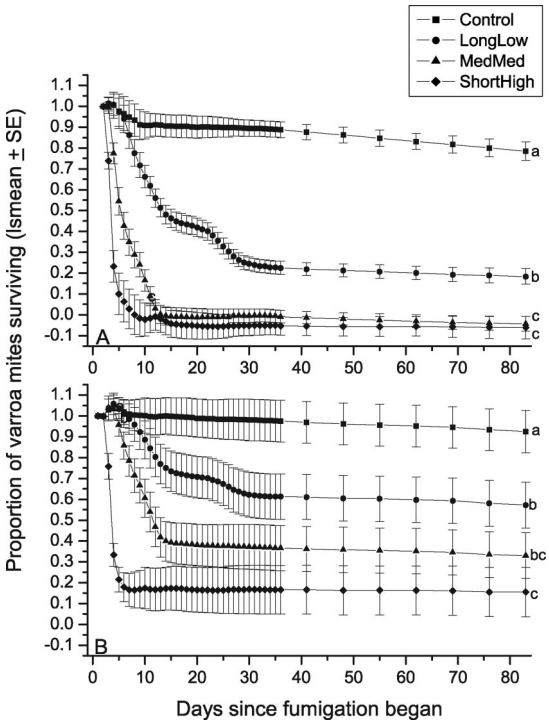

Fig. 2. Mean proportion of varroa mites (±SE) surviving over the course of the experiment in colonies from beekeeper UM (A) or AF (B) in rooms that were untreated (control), or received a long-term low concentration (LongLow), a medium-term medium concentration (MedMed) or a short-term high concentration (ShortHigh) of formic acid. Means followed by the same letter are not significantly different from each other ($P > 0.05$; Duncan's multiple range test). UM colonies contained an average of 347 ± 51 varroa mites, whereas AF colonies contained an average of 701 ± 133 varroa mites.

Queen Bee and Colony Mortality. The proportion of queens killed during fumigation differed between treatment rooms ($P < 0.001$) with zero of 20 queens dying in the control room, zero of 21 dying in the long-term low-concentration treatment, five of 21 queens dying in the medium-term medium-concentration treatment, and seven of 21 queens dying in the short-term high-concentration treatment (Fig. 4). Significantly more queens were killed in the short-term high concentration ($P < 0.01$) and medium-term medium concentration ($P < 0.05$) than in the control. Two queens were killed in the control room; one before the start of fumigation and another several weeks after fumigation ceased.

Treatment room did not significantly affect colony survival as estimated on 17 April, 9 d after the colonies were moved outdoors ($P > 0.05$). However, there was a significant effect of treatment room on the queen state of surviving colonies ($P < 0.01$; Fig. 4). Queenless colonies were successfully requeened in the spring after treatment.

Varroa Mite Mean Abundance. The change in mean abundance of varroa mites over the course of the experiment was affected by formic acid fumigation as indicated by a significant treatment room × exposure time interaction ($F = 11.36$; df = 3, 66; $P < 0.0001$; Fig. 5). All three acid treatments reduced the mean abundance of varroa mites relative to untreated controls.

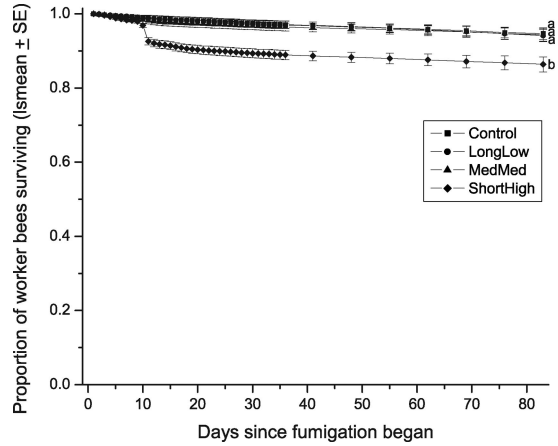

Fig. 3. Mean proportion of worker bees (±SE) surviving over the course of the experiment in rooms that were untreated (control; $n = 19$ hives) or received a long-term low concentration (LongLow; $n = 20$ hives), a medium-term medium concentration (MedMed; $n = 18$ hives), or a short-term high concentration (ShortHigh; $n = 13$ hives) of formic acid. Means followed by the same letter are not significantly different from each other ($P > 0.05$; Duncan's multiple range test).

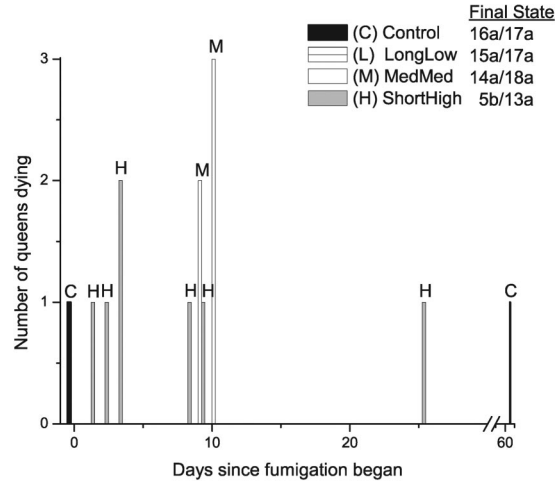

Fig. 4. Number of queens dying and dropping onto white boards over the course of the experiment in rooms that were untreated (control) or received a long-term low concentration (LongLow), a medium-term medium concentration (MedMed), or a short-term high concentration (ShortHigh) of formic acid. $n = 21$ colonies for each treatment. Final state refers to the number of queenright colonies on 17 April out of the total number of colonies in each treatment room that survived. Numbers followed by the same letter are not significantly different from the control ($P < 0.05$; Fisher's exact test).

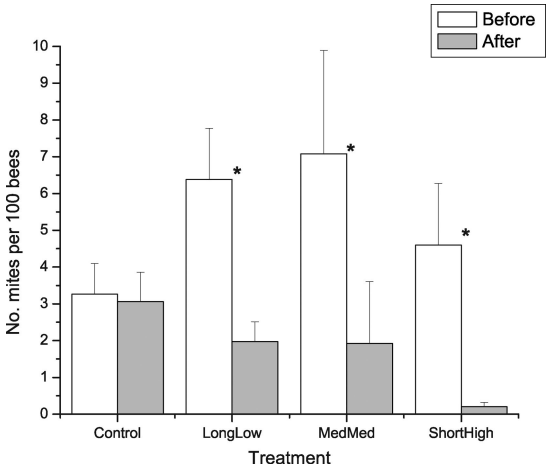

Fig. 5. Mean abundance of varroa mites (±SE) measured before and after wintering in colories that were untreated (control; $n = 19$ colonies), or received a long-term low concentration (LongLow; $n = 20$), a medium-term medium concentration (MedMed; $n = 18$), or a short-term high concentration (ShortHigh; $n = 13$) of formic acid. An asterisk indicates a significant treatment × exposure time interaction for each treatment compared with the control ($P \leq 0.01$).

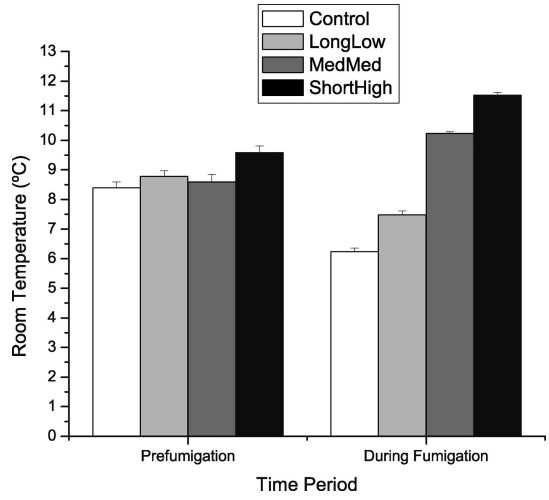

Fig. 7. Mean temperature (±SE) in each experimental treatment room from 2 to 11 January (prefumigation) and for the duration of fumigation in experimental rooms containing 21 colonies that were untreated (control; 27 d), or received a long-term low concentration (LongLow; 27 d), a medium-term medium concentration (MedMed; 10 d), or a short-term high concentration (ShortHigh; 9 d) of formic acid. Each bar represents measurements at three locations in each room measured every 30 min of the measurement period.

Honey Bee Population Size. At the start of fumigation, colonies from beekeeper AF had significantly larger populations than those from UM (AF;: 15,608 ± 1,239 bees; UM: 11,017 ± 1,174 bees; $F = 6.50$; df = 1, 49; $P = 0.01$). The change in the honey bee population size over winter was affected by formic acid fumigation as indicated by a significant treatment room × exposure time interaction ($F = 12.11$; df = 3, 66; $P < 0.001$; Fig. 6). The bee population in the short-term high concentration was reduced significantly more than in the control ($F = 22.91$; df = 1, 66; $P < 0.001$). Treatment room had no effect on the total brood area (mean 73 ± 11 cm^2; $F = 0.87$; df = 3, 30; $P > 0.05$), the capped brood area (mean 39 ± 6 cm^2; $F = 0.73$; df = 3, 30; $P > 0.05$), or the uncapped brood area (mean 34 ± 8 cm^2; $F = 0.56$; df = 3, 30; $P > 0.05$) of surviving colonies.

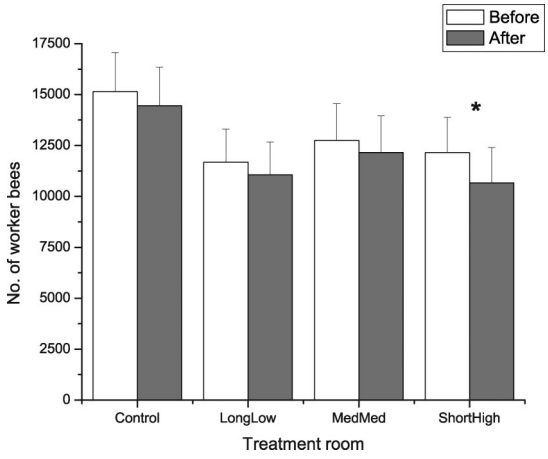

Fig. 6. Mean honey bee population size (±SE) measured before and after wintering in colonies that were untreated (control; $n = 19$) or received a long-term low concentration (LongLow; $n = 20$), a medium-term medium concentration (MedMed; $n = 18$), or a short-term high concentration (ShortHigh; $n = 13$) of formic acid. An asterisk indicates a significant treatment × exposure time interaction for each treatment compared with the control ($P < 0.05$).

Hive Weight. Treatment room did not affect hive weight loss as indicated by a nonsignificant treatment room × exposure time interaction ($F = 0.32$; df = 3, 60; $P > 0.05$). Before entering the wintering building, hives averaged 36.2 ± 0.5 kg, whereas after leaving the building they averaged 27.0 ± 0.4 kg. Before winter, colonies from beekeeper AF were heavier than those from UM (AF, 40.3 ± 0.5 kg; UM, 33.7 ± 0.5 kg; $F = 47.02$; df = 1, 62; $P < 0.0001$).

Room Temperature and Humidity. Mean temperature measurements for each treatment room are shown in Fig. 7. Before fumigation, there was no apparent difference in room temperature. However, during the first 9 d of fumigation, when all three treatment rooms were being fumigated, room temperature was higher in the acid-treated rooms than in the control room. Rooms with higher concentrations of acid tended to have higher temperatures and humidities. During the 27-d sampling period, the control room temperature ranged from 1.2 to 11.0°C, whereas the absolute humidity averaged 2.4 ± 0.0 g/m^3 (range 1.4–4.4 g/m^3) and the long-term low-concentration treatment ranged from 2.0 to 11.8°C and averaged 1.9 ± 0.0 g/m^3 (range 1.4–2.9 g/m^3). During the 10 d of fumigation in the medium-term medium-concen-

tration treatment, the temperature ranged from 7.8 to 17.9°C, whereas the absolute humidity averaged 5.9 ± 0.0 g/m^3 (range 3.7–7.4 g/m^3). During the 9 d of fumigation in the short-term high-concentration treatment, the temperature ranged from 5.8 to 14.9°C, whereas the absolute humidity averaged 7.0 ± 0.1 g/m^3 (range 3.7–10.5 g/m^3).

Discussion

Indoor winter fumigation with a CT product of ≈471 ppm*d of formic acid in room air significantly increased varroa mite mortality at all three exposure times. However, when this CT product was reached in 9 or 10 d, it was detrimental to worker bee survival, queen bee survival, or both. The best combination of formic acid concentration and exposure time was the long-term low concentration, which effectively controlled varroa mites without increasing worker or queen bee mortality.

The rate of varroa mite mortality in colonies from beekeeper UM was greater than that in colonies from beekeeper AF. The reason for this could not be determined from our experiment. The source of the mites, size of the colonies, and weight of hives all differed between beekeepers. AF colonies contained varroa mites that tested positive for fluvalinate resistance in the fall by the Pettis test (Pettis et al. 1998), whereas mites in colonies from UM were susceptible (unpublished data). The mites' resistance to fluvalinate in AF colonies may have affected their susceptibility to formic acid, but cross-resistance between these two chemicals has not been reported previously. In addition, AF colonies were heavier at the start of the experiment and had larger bee populations than those from UM. Underwood (2005) showed that cluster size, but not hive weight, is negatively correlated with in-hive CT product during formic acid fumigation and that mite mortality is related to this CT product. Because AF colonies had larger worker populations, they may have been exposed to lower concentrations of formic acid, resulting in lower efficacy during fumigation. Because all hives were not monitored for formic acid concentration in our experiment, it is not known which of these factors, if any, is the primary cause of the difference in varroa mite mortality rates between the two beekeepers' colonies.

Worker bee mortality was higher in colonies in the short-term high-concentration treatment than in untreated colonies, as was planned as part of our experimental design. This did not translate into a significant increase in colony mortality, but it did significantly affect the change in the size of the worker bee population. High worker mortality occurred on the 10th day of fumigation with a mean formic acid concentration of 52.5 ± 3.6 ppm in room air. However, after fumigation ceased in that treatment room, mortality rates of workers returned to the same rates that were found in all treatment rooms. Worker mortality in the rooms with lower concentrations of formic acid, but a similar CT product, did not differ from the control.

In the short-term high-concentration and the medium-term medium-concentration treatments, 33 and 24% of queens were killed as a result of fumigation, respectively. In contrast, despite the realization of an equivalent CT product in the room air in the long-term low-concentration treatment, no queens were found in white board samples. The queens that died in the short-term high-concentration treatment did so sporadically, with no identifiable temporal pattern. In contrast, the queens that dropped in the medium-term medium-concentration treatment did so in the last 2 d of fumigation. Because the air in only three hives per treatment room was sampled for formic acid concentration, we could not determine the relationship between queen loss and in-hive formic acid concentration in this experiment. However, Underwood (2005) showed that queen loss is associated with peak formic acid concentrations above 20 ppm in the hive. Queen loss in this experiment occurred in the final 2 d of fumigation in the medium-term medium-concentration treatment when the mean in-hive formic acid concentration was above 30 ppm. This may explain why queen loss occurred later in the treatment period in that treatment, but does not explain why the same thing did not happen in the short-term high-concentration treatment.

The relatively high temperatures during fumigation in this study also may have increased queen loss. It has been speculated that a combination of high temperatures and high concentrations of formic acid may contribute to queen loss (VonPosern 1988, Underwood 2005). The ventilation rate in experimental rooms in this study was held constant at 0.4 liters/s/m^3 to maintain consistent formic acid levels in the room air. Therefore, room temperatures averaged 7.5–11.5°C in fumigated treatment rooms, which is not typical during indoor wintering. Beekeepers generally use ventilation to keep the room temperature near 5°C (Gruszka 1998). Underwood (2005) showed that allowing ventilation rates to increase when room temperature exceeds 6°C can drastically reduce queen loss even when high concentrations of formic acid are used. Studies of how ventilation affects the in-hive CT product for long-term or low-concentration fumigation regimes are needed to determine efficacy of treatment under these conditions.

During this study, the CT product was based on room air measurements. However, hive air measurements showed that there is variation in formic acid concentration among hives in the same treatment room and that in-hive concentrations are much different from those in room air, as has been seen in previous studies (Underwood and Currie 2004, Underwood 2005). In-hive concentration has been shown to significantly influence queen and varroa mite mortality and should be used instead of the room air concentration to determine the CT product to which the bees are exposed (Underwood 2005). Future work on low-concentration fumigation should monitor varroa mite mortality and formic acid concentration in every exposed colony to determine the

cumulative concentration–mortality relationship for this treatment regime.

Both short-term high-concentration and medium-term medium-concentration fumigation with formic acid killed varroa mites, with averages of 93 and 83% mortality, respectively, but both treatments also were associated with an increase in mortality of worker bees, queen bees, or both. Long-term low-concentration fumigation had lower efficacy (60% varroa mite mortality), but it did not increase worker or queen bee mortality. A long-term fumigation with a formic acid concentration between the low and medium concentrations tested in this study may provide good efficacy while preventing queen loss. Efficacy varied with beekeeper within each treatment room, indicating that colony conditions and/or the source of varroa mites may affect fumigation results and should be considered when determining the appropriate combination of concentration and exposure time required. This study is one in a series of experiments designed to determine the most efficacious treatment regime that also minimizes harm to honey bees during indoor winter fumigation with formic acid. Further work is required before specific control recommendations can be made for the fumigation of commercial wintering buildings.

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