

**THE USE OF FORMIC ACID FOR CONTROL OF *VARROA DESTRUCTOR*
ANDERSON AND TRUEMAN AND OTHER PESTS IN OVERWINTERING
HONEY BEE, *APIS MELLIFERA* L., COLONIES**

BY

ROBYN M. UNDERWOOD

A Thesis presented to
the Faculty of Graduate Studies
in partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department of Entomology

University of Manitoba

Winnipeg, Manitoba

© Robyn M. Underwood, August 2005



Library and
Archives Canada

Bibliothèque et
Archives Canada

0-494-08805-2

Published Heritage
Branch

Direction du
Patrimoine de l'édition

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file *Votre référence*

ISBN:

Our file *Notre référence*

ISBN:

NOTICE:

The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protègent cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.


Canada

THE UNIVERSITY OF MANITOBA
FACULTY OF GRADUATE STUDIES

COPYRIGHT PERMISSION

**The Use of Formic Acid for Control of *Varroa destructor* Anderson and Trueman and Other
Pests in Overwintering Honey Bee, *Apis mellifera* L., Colonies**

BY

Robyn M. Underwood

**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of
Manitoba in partial fulfillment of the requirement of the degree**

Of

Doctor of Philosophy

Robyn M. Underwood © 2005

Permission has been granted to the Library of the University of Manitoba to lend or sell copies of this thesis/practicum, to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film, and to University Microfilms Inc. to publish an abstract of this thesis/practicum.

This reproduction or copy of this thesis has been made available by authority of the copyright owner solely for the purpose of private study and research, and may only be reproduced and copied as permitted by copyright laws or with express written authorization from the copyright owner.

Dedicated to my husband,
Todd J. Underwood,
for his untiring encouragement and support.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. R. W. Currie for his constant support. His knowledge, patience, and reassurance were essential throughout my time at the University of Manitoba. I would also like to thank the remaining members of my committee, Dr. L. C. Graham, Dr. N. J. Holliday, and Dr. N. D. G. White for their advice.

My husband, Dr. Todd J. Underwood, deserves a special thank you. His unconditional love and support sustained me throughout the process of completing this degree. If not for him, I would have lived these 7 years in an apartment piled with dirty dishes, inches of dust, and endless clutter. His love kept me sane. He also gave me my beautiful daughter, Sadie, whose smiles bring joy to my heart every day.

I would also like to thank L. Babey, L. Baspaly, K. S. Hemachandra, D. Holder, N. Froese, E. L. Klymochko, K. Pohl, G. Reinfelds, S. Surowiec, T. Teetaert, M. A. Tulp, J. Uddin, C. Wytrykush, and D. Wytrykush, who provided technical support over the years. In particular, Lisa Babey were an integral part of the research process. Her technical and emotional support was invaluable. I can honestly say she is the kindest, most giving person I have ever met.

I thank beekeepers C. Bartel, A. Bergman, W. Bygarski Jr., B. Clark, T. Fehr, A. Friesen, R. Futros, P. Gregory, C. Isaac, D. Kitson, M. Malyon, L. Peters, C. Polcyn, J. Reimer, J. Warburton, and the University of Manitoba for donating honey bee colonies and P. Veldhius for donating colonies and providing logistical support. This work could not have been done without their generosity. In addition, D. Dixon, R. Lafreniere, D. Ostermann, D. Treble, and D. Vanderwel provided logistical support and Dr. G. Crow provided statistical advice.

Funding for this research was a result of grants to Dr. R. W. Currie from the Saskatchewan Agri-Food Innovation Fund, the Manitoba Beekeepers' Association, the Canadian Bee Research Fund, and the Canada-Manitoba Agri-food Research and Development Initiative (ARDI). I also received scholarships from the American Beekeeping Federation, the Canadian Association of Professional Apiculturists, the Canadian Society of Agronomy, and the Entomological Society of Manitoba.

I hereby declare that I am the sole author of this thesis.

I authorize the University of Manitoba to lend this thesis to other institutions or individuals for the purpose of scholarly research.

Robyn M. Underwood

I further authorize the University of Manitoba to reproduce this thesis by photocopying or by other means, in whole or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Robyn M. Underwood

TABLE OF CONTENTS

ACKNOWLEDGMENTS	III
LIST OF FIGURES	XV
LIST OF TABLES	XX
ABSTRACT.....	1
GENERAL INTRODUCTION.....	3
LITERATURE REVIEW	6
INTRODUCTION	6
VARROA MITE REPRODUCTION AND HARM TO HONEY BEES.....	7
FORMIC ACID.....	15
<i>Methods of use</i>	16
<i>Effect of treatment on honey bees</i>	19
RATIONALE OF THIS STUDY	21
CHAPTER 1. THE EFFECTS OF TEMPERATURE AND CONCENTRATION OF FORMIC ACID ON TREATMENT EFFICACY AGAINST THE HONEY BEE, <i>APIS MELLIFERA</i> L. (HYMENOPTERA: APIDAE), PARASITE <i>VARROA DESTRUCTOR</i> ANDERSON AND TRUEMAN (ACARI: VARROIDAE).....	23
ABSTRACT.....	23
INTRODUCTION	25
METHODS AND MATERIALS	27

<i>Experimental Setup</i>	27
<i>Formic Acid Concentration</i>	29
<i>Data Analysis</i>	30
RESULTS	30
<i>Survival</i>	30
<i>CT Products</i>	31
DISCUSSION	32

CHAPTER 2. INDOOR WINTER FUMIGATION OF *APIS MELLIFERA* L.

(HYMENOPTERA: APIDAE) COLONIES INFESTED WITH *VARROA*

***DESTRUCTOR* ANDERSON AND TRUEMAN (ACARI: VARROIDAE) WITH**

FORMIC ACID IS A POTENTIAL CONTROL ALTERNATIVE IN NORTHERN

CLIMATES

ABSTRACT	40
INTRODUCTION	42
MATERIALS AND METHODS	44
<i>Time line of events</i>	44
<i>Pre-treatment sampling</i>	44
<i>Hive setup</i>	46
<i>Room temperature and airflow</i>	47
<i>Treatment</i>	48
<i>Formic Acid Sampling</i>	49
<i>Post-treatment</i>	50
<i>Statistical analysis</i>	51

RESULTS	52
<i>Formic acid concentration</i>	52
<i>V. destructor mortality</i>	53
<i>Worker bee mortality</i>	53
<i>Queen bee mortality</i>	54
<i>Mean V. destructor abundance</i>	54
<i>Mean N. apis spore abundance</i>	54
<i>A. woodi prevalence</i>	55
<i>Colony weight</i>	55
<i>Cluster size</i>	55
<i>Temperature</i>	56
<i>Colony survival</i>	56
DISCUSSION	56

CHAPTER 3. INDOOR WINTER FUMIGATION OF HONEY BEES WITH FORMIC ACID: A LOW TEMPERATURE CUMULATIVE CONCENTRATION- RESPONSE FOR VARROA MITES AND A POSSIBLE QUEEN LOSS

PREVENTION STRATEGY	70
ABSTRACT.....	70
INTRODUCTION	71
MATERIALS AND METHODS	73
<i>Experimental treatment rooms and general fumigation methods</i>	73
<i>Experiment 1</i>	75
<i>Experiment 2</i>	76

<i>Statistical Analysis</i>	78
RESULTS	80
<i>Experiment 1</i>	80
<i>Experiment 2</i>	81
DISCUSSION	84

**CHAPTER 4. WHAT IS THE MOST EFFICACIOUS CONCENTRATION X
TIME COMBINATION DURING INDOOR WINTER FUMIGATION WITH
FORMIC ACID FOR MAXIMIZING VARROA MITE CONTROL WHILE
MINIMIZING THE EFFECT ON WORKER AND QUEEN HONEY BEES?..... 97**

ABSTRACT	97
INTRODUCTION	99
MATERIALS AND METHODS	100
<i>Prior to experimentation</i>	100
<i>Fumigation</i>	101
<i>Following fumigation</i>	105
<i>Statistical analysis</i>	106
RESULTS	107
<i>Formic acid concentration</i>	107
<i>Varroa mite daily mortality</i>	108
<i>Worker bee daily mortality</i>	108
<i>Queen bee and colony mortality</i>	109
<i>Varroa mite mean abundance</i>	110
<i>Honey bee population size</i>	110

<i>Hive weight</i>	110
<i>Room temperature and humidity</i>	111
DISCUSSION	111

CHAPTER 5. LONG-TERM LOW CONCENTRATION FORMIC ACID

FUMIGATION FOR CONTROL OF VARROA MITES, *VARROA DESTRUCTOR*, IN INDOOR WINTERING HONEY BEE, *APIS MELLIFERA*, COLONIES 125

ABSTRACT	125
INTRODUCTION	127
MATERIALS AND METHODS	129
<i>Colony preparation</i>	129
<i>Fumigation</i>	130
<i>Formic acid concentration measurements</i>	132
<i>Final assessment</i>	133
<i>Honey analysis</i>	133
<i>Statistical analysis</i>	135
RESULTS	137
<i>Formic acid concentration</i>	137
<i>Varroa mite daily mortality</i>	138
<i>Worker bee daily mortality</i>	139
<i>Queen bee and colony mortality</i>	140
<i>Varroa mite mean abundance</i>	140
<i>Worker bee population size</i>	141
<i>Hive weight</i>	141

<i>Room temperature and humidity</i>	141
<i>Formic acid concentration in honey</i>	141
DISCUSSION	142

CHAPTER 6. INDOOR WINTER FUMIGATION WITH FORMIC ACID FOR CONTROL OF THE HONEY BEE TRACHEAL MITE, *ACARAPIS WOODI*

(RENNIE), AND NOSEMA DISEASE, *NOSEMA APIS* ZANDER. 156

ABSTRACT	156
INTRODUCTION	157
MATERIALS AND METHODS	159
<i>Experimental treatment rooms and general fumigation methods</i>	159
<i>Pre- and post-treatment sampling</i>	160
<i>Experiment 1</i>	160
<i>Experiment 2</i>	161
<i>Experiment 3</i>	163
<i>Statistical Analysis</i>	163
RESULTS	164
<i>Tracheal mite prevalence</i>	164
<i>Tracheal mite survival</i>	165
<i>Nosema disease</i>	165
DISCUSSION	165
<i>Tracheal mites</i>	166
<i>Nosema disease</i>	167

CHAPTER 7. INDOOR WINTER FUMIGATION WITH FORMIC ACID DOES NOT HAVE A LONG-TERM EFFECT ON HONEY BEE QUEEN

PERFORMANCE.....	175
ABSTRACT	175
INTRODUCTION	177
MATERIALS AND METHODS	178
<i>Experiment 1</i>	178
<i>Experiment 2</i>	179
<i>Determination of long-term effects</i>	180
<i>Statistical Analysis</i>	181
RESULTS	181
DISCUSSION	182

CHAPTER 8. FORMIC ACID IN BEE FEED: ITS EFFECT ON HONEY BEE QUEEN PHEROMONES AND ATTENDANCE BY WORKERS.

ABSTRACT	187
INTRODUCTION	189
MATERIALS AND METHODS	190
<i>Bioassay</i>	190
<i>Pheromone analysis</i>	191
<i>Statistical analysis</i>	193
RESULTS	194
<i>Bioassay</i>	194
<i>Pheromone analysis</i>	194

DISCUSSION	195
GENERAL DISCUSSION	204
EFFICACY AGAINST PESTS AND DISEASES.....	204
<i>Varroa mites</i>	204
<i>Tracheal mites</i>	209
<i>Nosema spores</i>	210
FACTORS AFFECTING FORMIC ACID CONCENTRATION	211
EFFECTS ON WORKERS, QUEENS, AND QUEEN PERFORMANCE	216
<i>Worker bees</i>	216
<i>Queen bees</i>	218
<i>Queen performance</i>	222
SUMMARY	223
THE NEED FOR FUTURE WORK	225
LITERATURE CITED	227

LIST OF FIGURES

Figure 1.1. Varroa mite survival at 5°C (a), 15°C (b), 25°C (c), and 35°C (d) over the duration of the experiment.....	37
Figure 1.2. Worker bee survival at 5°C (a), 15°C (b), 25°C (c), and 35°C (d) over the duration of the experiment.....	38
Figure 1.3. CT ₅₀ products for bees and mites at 5°C (a), 15°C (b), 25°C (c), and 35°C (d).....	39
Figure 2.1. Setup of a treatment room..	63
Figure 2.2. Air mixing chamber.....	64
Figure 2.3. Mean formic acid concentration (\pm SE) in the bottom, middle, and top sample sites of hives stacked three high.....	65
Figure 2.4. Mean formic acid concentration in air (\pm SE) of treatment rooms and from the center, and edge of the cluster within hives during (0-48 h) and after (72 h) fumigation.....	66
Figure 2.5. Mean number of varroa mites (A) or worker honey bees (B) falling each day (\pm SE) from colonies that were untreated or treated with a low, medium, or high concentration of formic acid for 48 h.....	67
Figure 2.6. Varroa mite mean abundance (\pm SE) (A), nosema spore mean abundance (millions of spores per bee \pm SE) (B), or tracheal mite prevalence (no. of infested bees per 100 bees \pm SE) (C) measured before and after wintering in colonies that were untreated or treated with a low, medium, or high concentration of formic acid for 48 h.....	69

Figure 3.1. Mean (\pm SE) formic acid concentration in the high concentration spike formic acid treatment (A) and the constant high-concentration treatment (B) in room and hive air in experiment 1..	90
Figure 3.2. Varroa mite mortality (mean \pm SE) over time in colonies fumigated with formic acid for 5 days in experiment 1.....	91
Figure 3.3. Concentration-response curve for varroa mites in colonies fumigated with formic acid for 5 days in experiment 1.....	92
Figure 3.4. Formic acid concentration in the minimum-ventilation fumigated room (A) and variable-ventilation fumigated room (B) in experiment 2.	93
Figure 3.5. Mean in-hive CT product of formic acid (\pm SE) in hives stacked in acid treatment rooms from the floor to the top of the stack in experiment 2..	94
Figure 3.6. Varroa mite mean abundance (\pm SE) measured before and after wintering in colonies that were unfumigated with minimum ventilation or variable ventilation or treated with a high concentration of formic acid with minimum ventilation or variable ventilation for 7 days.....	95
Figure 3.7. Timing of queen drop onto the white board in colonies that were unfumigated with minimum ventilation or variable ventilation or treated with a high concentration of formic acid with minimum ventilation or variable ventilation for 7 days.	96
Figure 4.1. Concentration of formic acid (mean \pm SE) in room and hive air during fumigation in rooms receiving a low concentration for 27 days (A), a medium concentration for 10 days (B) or a high concentration for 9 days (C).	116

Figure 4.2. Mean proportion of varroa mites (\pm SE) surviving over the course of the experiment in colonies from beekeeper UM (A) or AF (B) in rooms that were untreated, or received a long-term low concentration, a medium-term medium concentration or a short-term high concentration concentration of formic acid..... 118

Figure 4.3. Mean proportion of worker bees (\pm SE) surviving over the course of the experiment in rooms that were untreated, or received a long-term low concentration, a medium-term medium concentration or a short-term high concentration of formic acid..... 120

Figure 4.4. Number of queens dropping onto white boards over the course of the experiment in rooms that were untreated, or received a long-term low concentration, a medium-term medium concentration or a short-term high concentration.of formic acid..... 121

Figure 4.5. Mean abundance of varroa mites (\pm SE) measured before and after wintering in colonies that were untreated, or received a long-term low concentration, a medium-term medium concentration or a short-term high concentration.of formic acid..... 122

Figure 4.6. Mean honey bee population size (\pm SE) measured before and after wintering in colonies that were untreated, or received a long-term low concentration, a medium-term medium concentration or a short-term high concentration of formic acid..... 123

Figure 4.7. Mean temperature (\pm SE) in each experimental treatment room from 2 – 11 January (pre-fumigation) and for the duration of fumigation in experimental rooms containing 21 colonies that were untreated, or received a long-term low

concentration, a medium-term medium concentration or a short-term high concentration of formic acid.	124
Figure 5.1. Mean (\pm SE) formic acid concentration (ppm) in room and hive air in the variable ventilation 47-day treatment room (A), minimum ventilation 47-day treatment room (B), and variable ventilation 65-day treatment room (C).....	150
Figure 5.2. Mean in-hive CT product of formic acid (\pm SE) in hives stacked in all three acid-fumigated rooms from the floor to the top of the stack..	152
Figure 5.3. Varroa mite survival (weighted $\bar{x} \pm$ SE) over the course of the experiment in colonies in rooms that were untreated with variable ventilation, treated with formic acid for 47 days with variable ventilation, treated for 47 days with minimum ventilation, or treated for 65 days with variable ventilation. Means are weighted by the total number of varroa mites present at the start of fumigation..	153
Figure 5.4. Concentration-response curve for varroa mites in colonies treated with a long-term low concentration of formic acid for 47 or 65 days..	154
Figure 5.5. Formic acid concentration in capped (A) and uncapped (B) honey before and after exposure to 10 – 17 ppm formic acid in room air for 10, 20, 30 or 40 days. .	155
Figure 6.1. Tracheal mite prevalence (mean \pm SE) as measured by thoracic slices before and after formic acid fumigation in three separate indoor winter fumigation experiments.....	169
Figure 6.2. Proportion of tracheal mites alive (mean \pm SE) as measured by live dissection before and after formic acid fumigation in two separate indoor winter fumigation experiments.....	171

Figure 6.3. Nosema spore mean abundance (mean \pm SE) before and after formic acid fumigation in three separate indoor winter fumigation experiments.....	173
Figure 7.1. Capped brood area (mean \pm SE) for colonies unfumigated or treated with formic acid using a long-term low concentration, medium-term medium concentration or short-term high concentration during the winter of 2001-2002 in experiment 1.....	185
Figure 7.2. Honey production (mean \pm SE) during the summer of 2002 for colonies untreated or treated with formic acid during the winter of 2001-2002.....	186
Figure 8.1. Set-up of bioassay cages.....	200
Figure 8.2. The proportion of worker bees (mean \pm SE) attending the queen in a bioassay where bees were fed unadulterated sugar syrup or sugar syrup containing 100 ppm or 1,000 ppm formic acid in summer or winter	201
Figure 8.3. Pheromone quantity in honey bee queen body washes (mean \pm SE) from a bioassay where bees were fed unadulterated sugar syrup or sugar syrup containing 100 ppm or 1,000 ppm formic acid.....	202
Figure 8.4. Pheromone quantity in honey bee queen mandibular glands from a bioassay where bees were fed unadulterated sugar syrup or sugar syrup containing 100 ppm or 1,000 ppm formic acid.....	203

LIST OF TABLES

Table 2.1. Mean cluster ranking and hive weight (\pm SE) measured before and after wintering in colonies that were untreated or treated with a low, medium, or high concentration of formic acid for 48 h.....	62
Table 5.1. Average (mean \pm SE) daily varroa mite mortality rate, formic acid concentration in hive, temperature, and relative humidity in rooms that were untreated with variable ventilation, treated with formic acid for 47 days with variable ventilation, treated for 47 days with minimum ventilation, or treated for 65 days with variable ventilation.....	145
Table 5.2. Mean (\pm SE) honey bee population size, hive weight, and varroa mite mean abundance in colonies that were untreated or treated with a low concentration of formic acid with variable ventilation for 47 days, with minimum ventilation for 47 days, or with variable ventilation for 65 days.....	146
Table 8.1. Statistical analysis of pheromone gland quantity in body washes of queens in a bioassay where bees were fed unadulterated sugar syrup or sugar syrup containing 100 ppm or 1,000 ppm formic acid.....	195
Table 8.2. Statistical analysis of pheromone gland quantity in the mandibular glands of queens in a bioassay where bees were fed unadulterated sugar syrup or sugar syrup containing 100 ppm or 1,000 ppm formic acid.....	196

ABSTRACT

The varroa mite, *Varroa destructor* Anderson and Trueman, is a threat to the beekeeping industry. Using formic acid indoors in winter is one method for controlling this ectoparasite in honey bee, *Apis mellifera* L., colonies. This study consists of experiments conducted to determine the most efficacious treatment regime that also minimizes harm to honey bees.

Varroa mite mortality was significantly increased by formic acid fumigation, in a concentration-dependent manner. Long-term low concentration fumigation and short-term high concentration fumigation both provided good efficacy. Worker bee mortality was not increased by fumigation, but high concentration fumigation caused significant queen loss. Long-term low concentration fumigation or high concentration fumigation with step-wise temperature-dependent room ventilation prevented queen loss. There was no effect of treatment on any measure of performance of surviving queens during the summer following treatment.

The prevalence of tracheal mites was not affected by formic acid fumigation, however, tracheal mite mortality was significantly increased by formic acid fumigation. Nosema spore mean abundance was significantly suppressed by formic acid fumigation in only one of four experiments. However, in all experiments, nosema spore abundance was lower in treated than in untreated colonies.

During fumigation, the concentration of formic acid in hive air was consistently lower than that in room air possibly because honey absorbs the acid. Workers fed acid-laden sugar syrup in summer were less attentive to queens when fed 100 ppm formic acid, than when fed 0 or 1000 ppm formic. The quantity of each component of the

queen's pheromone blend was not affected by formic acid treatment. However, one component, methyl *p*-hydroxybenzoate, varied with season.

GENERAL INTRODUCTION

The varroa mite, *Varroa destructor* Anderson and Trueman is considered the most serious threat to the beekeeping industry. Because this ectoparasitic mite has become resistant to the pesticides most commonly used in North America, fluvalinate (e.g. Eischen, 1995; Lodesani et al., 1995; Milani, 1995; Colin et al., 1997; Elzen et al., 1998; Trouiller, 1998; Mozes-Koch et al., 2000; Floris et al., 2001) and coumaphos (e.g. Spreafico et al., 2001; Pettis, 2004), alternative control techniques are desirable and necessary. Formic acid, which was first used in Germany in 1980 (Ritter and Ruttner, 1980), is an organic acid that is ideal for use in honey bee, *Apis mellifera* L., colonies because it is inexpensive, readily available, and can effectively control varroa mites without harming bees if used properly. However, formic acid efficacy against varroa mites is variable, especially when used outdoors, due to variation in factors such as ambient temperatures (VonPosem, 1988; Feldlaufer et al., 1997; Calderone, 1999; Ostermann and Currie, 2004), size of the brood area (Eischen, 1998), and time of year (Maul et al., 1980). The research reported in this thesis focused on using formic acid as a fumigant indoors in winter. Indoor winter fumigation is advantageous because ambient temperatures can be controlled and varroa mites seldom escape treatment by entering brood cells: brood rearing is greatly reduced in winter. A review of the literature pertinent to this work is presented in the Literature Review.

Chapter 1 was a laboratory study of the effects of temperature and concentration of formic acid on both honey bee workers and varroa mites. The combination of formic acid concentration and exposure time that was most selective against the varroa mites was determined at various temperatures. The effect of temperature and formic acid

concentration on efficacy of treatment was examined. This chapter was published in *Experimental and Applied Acarology*, 2003, volume 29, pages 303-313.

Chapter 2 was a field study to assess the feasibility of indoor winter fumigation with formic acid. Full-size colonies were fumigated with various concentrations of formic acid for 48 h. Varroa mite mortality was monitored to determine the efficacy of each treatment. The effect of each treatment on queen bees, worker bees, tracheal mites, *Acarapis woodi* (Rennie), and nosema disease, *Nosema apis* Zander, was also examined. This chapter was published in the *Journal of Economic Entomology*, 2004, volume 97, pages 177-186.

Chapter 3 was a field study designed to determine the cumulative concentration-mortality relationship between formic acid and varroa mites during indoor winter fumigation with a high concentration of formic acid and the effect of room ventilation rate on queen loss during high concentration fumigation.

Chapter 4 was a field study designed to determine the most desirable combination of formic acid concentration and exposure time. Full-size honey bee colonies were exposed to either a short-term low concentration, medium-term medium concentration or short-term high concentration of formic acid until similar concentration x time products were realized. The effect of these treatments on queen bees, worker bees and varroa mites was examined.

Chapter 5 was a field study designed to determine the effect of a long-term low concentration of formic acid on queen bees, worker bees, and varroa mites. Room temperature in treatment rooms was intentionally or naturally increased for part of the fumigation period to determine the potential effect of an increase in room temperature on

varroa mite mortality and formic acid concentration. A cumulative concentration-mortality relationship for varroa mites and formic acid at a low concentration of formic acid was determined. In addition, the absorption of formic acid by capped and uncapped honey was examined.

Chapter 6 was a determination of the effect of formic acid fumigation indoors in winter on tracheal mites and nosema disease. The effect of treatments from chapters 3, 4 and 5 on the efficacy of formic acid fumigation for control of these pests was determined.

Chapter 7 was a field study designed to examine the long-term effects of winter fumigation on honey bee queens. Colonies from experiments described in chapters 3 and 4 whose queens survived treatment during the winter were followed through the subsequent summer. The effect of treatments on honey production, brood production and the frequency of queen supersedure was examined for one production season.

Chapter 8 was a laboratory study of the effect of formic acid in honey bee feed on queen honey bees. A laboratory bioassay was conducted in summer and in winter to determine the effect of acid-laden feed on the number of worker bees attending queens. The quantity of pheromone components on the queens' bodies and in their mandibular glands was also measured.

This study was an examination of the use of formic acid as a fumigant indoors in winter. The main objectives were to apply the concepts related to the principles of fumigation to formic acid fumigation of honey bees, to determine the feasibility of this method as a pest control technique, and to determine the treatment regime that causes the maximum varroa mite mortality while causing the minimum effect on honey bees.

LITERATURE REVIEW

INTRODUCTION

The varroa mite, *Varroa destructor* Anderson and Trueman, is considered the most serious honey bee, *Apis mellifera* L., pest in the world (Anderson and Trueman, 2000). There are several species of mites in the genus *Varroa* that are in the order Acari, the group Parasitiformes, the suborder Mesostigmata, and the family Varroidae (Delfinado and Baker, 1974; Borror et al., 1989; Anderson and Trueman, 2000). The varroa mites that were first found in colonies of *Apis mellifera* in Europe in 1958 (Anderson and Fuchs, 1998) were thought to be *Varroa jacobsoni* Oudemans (1904), which was described from specimens collected in colonies of the Asian hive bee, *Apis cerana* Fabricius in Java. Studies of differential reproductive success of varroa mites in various geographic locations led to the discovery of several haplotypes of varroa mites and eventually to the division of the varroa mite into two species, *Varroa jacobsoni* and *Varroa destructor* (Delfinado-Baker, 1988; Anderson and Fuchs, 1998; Anderson and Trueman, 2000). Anderson and Trueman (2000) showed that the species of varroa mite found on *Apis mellifera* is actually *Varroa destructor*, a highly virulent pest. This species comprises at least six haplotypes infesting *Apis cerana* in Asia, two of which are also found in colonies of *Apis mellifera* in the Americas (Anderson, 2000; Anderson and Trueman, 2000). These haplotypes differ in their virulence, with those found on *Apis mellifera* being the most destructive (Delfinado-Baker, 1988; Anderson and Fuchs, 1998). Papers published prior to 2000 from work conducted in Europe and North America that refer to *Varroa jacobsoni* on *Apis mellifera* were actually studies on *Varroa destructor* (Anderson and Trueman, 2000). For the remainder of this dissertation, I use

the name “varroa mite” to refer to *Varroa destructor* (DeGuzman et al., 1998; Anderson, 2000; Anderson and Trueman, 2000; Cobey, 2001).

It is believed that varroa mites transferred from Asian hive bees to European honey bees when they were kept together in Asia in the early 1900s (Ruttner, 1983; Crane, 1988). The European honey bees were then transported to the Ukraine where the mites were first reported damaging colonies in 1963 (Delfinado, 1963). Since that time, the mites have spread to every continent where bees are kept.

The varroa mite is an obligate parasite on *Apis* species (Eickwort, 1988) and requires immature honey bee larvae for food and reproduction, and adult workers for food and dispersal within and among hives. A colony infested with varroa mites that is not treated will collapse (die) within three to four years of the initial infestation (Rosenkranz and Engels, 1985). Prior to collapse, honey production is reduced and the pest may be spread to other colonies (Gatien and Currie, 1995). This literature review will focus on the varroa mite’s biology and life cycle, the effect it has on honey bee colonies and the potential use of formic acid as a control measure for the mite.

VARROA MITE REPRODUCTION AND HARM TO HONEY BEES

Throughout their life, adult female varroa mites are usually found on adult or immature honey bees and spend little time away from their host. They preferentially attach themselves to nurse bees (Kraus, 1993), which tend the bee larvae during the brood rearing season. They use their proboscis-like mouthparts to pierce the intersegmental membranes of the abdomen and feed on the bee’s hemolymph (DeLillo et al., 2001). During brood rearing periods, mites remain on adult worker bees for 1 - 10 days (Boot et

al., 1993). However, when bees are not reproducing, the mites must remain on adult bees for substantially longer periods of time (Korpela et al., 1992). When ready to reproduce, the female mite enters a cell containing a fifth instar worker or drone larva (Ifantidis, 1988) at about 20 or 40 hours prior to cell capping, respectively (Boot et al., 1992).

After the cell containing the immature bee and adult female varroa mite is capped, the mite, known as the foundress, feeds on the bee (Ritter, 1981). She then lays eggs, approximately one every 26 - 32 hours over 5 days (Martin, 1994). The first egg is unfertilized and develops into a male and all subsequent eggs are fertilized and develop into females (Rehm and Ritter, 1989; Martin, 1994; Donzé and Guerin, 1997; Donzé et al., 1998). The developing mite family feeds from an incision in the bee pupa's abdominal cuticle, which was made by the foundress (Donzé and Guerin, 1994; Donzé et al., 1998). When the young mites reach sexual maturity, at 6 - 7 days of age for males and 8 - 10 days of age for females (Ritter, 1981; DeJong et al., 1982), they mate with each other (Donzé et al., 1998). When the bee reaches maturity, it chews through the wax capping and emerges from the cell, bringing with it all female mites that have reached maturity and leaving male and immature mites in the cell to die (Martin, 1994). Mated female mites that emerge from the cell go on to continue the reproductive cycle, as does the foundress, going through an average of 2 - 3 reproductive cycles in a lifetime (Fries et al., 1994; Martin and Kemp, 1997). Upon emergence from the cell, mites quickly find a new adult bee host (LeConte and Arnold, 1987).

Mite populations grow rapidly. An initial infestation of 5 - 8 female mites in a colony can increase by 100-fold in a single summer (Fries et al., 1991). Therefore, the time between the initial infestation and the damage to the colony is rather short. Varroa

mites can damage bees making them underweight, anemic, deformed, short-lived, and susceptible to other diseases.

Varroa mites cause damage to worker honey bee pupae. The most remarkable effect is the reduction of weight. One female mite can reduce a worker bee's weight at emergence to 9.6 % below average weight (Schneider and Drescher, 1987). Each additional foundress mite reproducing in the cell with the bee causes further weight reduction (DeJong et al., 1982; Schneider and Drescher, 1987; Schatton-Gadelmayer and Engels, 1988). Infested bees do not compensate for this weight loss after emergence even when they are placed in healthy, uninfested colonies (Schneider and Drescher, 1987).

Young worker adults infested with varroa mites have fewer hemocytes and lower hemolymph protein levels than uninfested workers (Weinberg and Madel, 1985; Schatton-Gadelmayer and Engels, 1988; Wienands and Madel, 1988; Amdam et al., 2004). Total protein concentration in honey bee hemolymph also decreases as the number of mites in the cell increases (Glinski and Jarosz, 1984; Schatton-Gadelmayer and Engels, 1988). Tolerance to pesticides is reduced in infested honey bee colonies (Drescher and Schneider, 1988) and their immune system is suppressed (Sammataro, 1997).

Varroa mite infestation reduces the longevity of honey bees by 34 – 40 % (DeJong and DeJong, 1983; Kovac and Crailsheim, 1988). On average, worker bees that develop with one foundress mite live 18.1 days, whereas uninfested bees live 27.6 days (DeJong and DeJong, 1983). With each additional foundress in the cell with the developing bee, worker lifespan decreases even more (DeJong and DeJong, 1983).

Honey bee workers emerging from mite-infested cells often have deformed wings. The frequency of deformity is correlated with the level of infestation (Schatton-Gademayer and Engels, 1988; Koch and Ritter, 1991). Several explanations for this effect of infestation have been suggested. The first is that the loss of hemolymph caused by the feeding of the mites reduces the bee's ability to expand its wings upon eclosion. This is supported by the work of Koch and Ritter (1991) who experimentally removed hemolymph from developing pupae and found that similar wing deformation results. A second cause is the reduction in brood care that occurs in mite-infested colonies. Schneider and Drescher (1987) found that the hypopharyngeal glands of nurse bees that are infested with varroa mites as pupae are poorly developed and provide insufficient nourishment for the brood. Further, undernourished brood emerges underweight and with deformed wings even though they are not infested directly with mites (Schneider, 1987). A third cause for deformed wings in infested bees may be enhanced transmission of deformed wing virus, which is thought to be spread among bees by varroa mites (Martin, 2001).

Varroa mite infestation also negatively affects drones. Drones emerging from mite-infested cells have reduced body weight and are sometimes deformed (Ritter, 1988; Rinderer et al., 1999; Collins and Pettis, 2001). Studies on the effect of varroa mites on drone reproduction have yielded contradictory results. It has been shown that drones infested as pupae have smaller seminal vesicle and mucus gland weights and fewer spermatozoa than uninfested drones (Ritter, 1988; Rinderer et al., 1999). However, Collins and Pettis (2001) found no difference in the number of live and dead sperm, the volume of semen, or the concentration of sperm in mature drones emerging from cells

that are infested with varroa mites or uninfested. The contradiction in results is likely due to the effect of varroa mites on drone survival which affects the results of studies requiring mature drones. The number of drones surviving to sexual maturity (12 days post-emergence) is significantly reduced by varroa mite infestation (Rinderer et al., 1999; Collins and Pettis, 2001), but infested drones that survive to sexual maturity are as capable of successful mating as uninfested drones (Sylvester et al., 1999). Thus, queen producers need to rear greater numbers of drones to obtain successful matings if their colonies are infested with varroa mites.

Varroa mites also harm honey bees by spreading diseases. The spores of a common honey bee fungal disease, chalkbrood or *Ascosphaera apis* (Maassen ex Claussen), are carried by varroa mites (Liu, 1996). In addition, Strick and Madel (1988) found that varroa mites are capable of transmitting the pathogenic bacterium *Hafnia alvei* Moller. It is thought that bacteria are able to enter the bee through the feeding wounds caused by the mites (Strick and Madel, 1988; Sammataro, 1997). Presence of this bacterium in the blood is highly correlated with septic infections in honey bees (Strick and Madel, 1988). Varroa mites are also thought to spread honey bee viruses (Ball, 1985; Ball and Allen, 1988; Liu and Ritter, 1988; Pugh et al., 1992; Chen et al., 2004). Some pathogens, such as acute paralysis virus, have been found to be at levels high enough to be lethal only when varroa mites are present in the colony (Ball and Allen, 1988; Sammataro, 1997). Other viruses associated with colony death in mite-infested colonies are deformed wing virus, slow paralysis virus, cloudy wing virus (Martin et al., 1998) and Kashmir bee virus (Sammataro, 1997; Chen et al., 2004).

Varroa mites are a serious threat to honey bees and the beekeeping industry. Highly infested bees are anemic, below normal weight, short-lived and sometimes cannot fly. Fortunately, early diagnosis of the problem can allow treatment to prevent the high infestations that cause economic damage to colonies. Varroa mite infestation levels need to be monitored to allow the beekeeper to make informed decisions about when control measures are necessary. There are several methods commonly used to detect varroa mite infestations. The first is the sticky board method. A white board covered with a sticky substance such as petroleum jelly or cooking spray is placed on the bottom board of the hive under a protective screen that allows mites, but not bees, to fall through. The number of mites found on the sticky board after a period of time (e.g. 48 hours) is used to estimate the mite population in the colony (Calatayud and Verdu, 1995; Martin and Hogarth, 1994; Marcangeli, 1997). Another sampling method is the ether roll method. After 200-300 nurse bees are collected in a jar, automobile starter fluid is sprayed into the jar, which is then rolled and shaken for about a minute (Herbert et al., 1989). The mites are dislodged from the bees and stick to the inside of the jar where they can be counted and used to estimate the mean abundance of mites in the colony. The mean abundance is the average number of mites per bee (Bush et al., 1997), but is often expressed as the number of mites per 100 bees for ease of interpretation. Another method for estimating the mean abundance of varroa mites in a honey bee colony is the alcohol wash method. This is similar to the ether roll method, where a known number of bees is collected, washed in 70 % alcohol and sieved to estimate the mean abundance of varroa mites (DeJong et al., 1982; Lupo and Gerling, 1990). Decisions about treatment of colonies

based on economic thresholds are usually based on samples collected by one of these methods.

The goal of an economic threshold is to predict when treatments should be applied to keep the pest population below a level that causes economic damage to its host. Economic damage occurs when the value of the damage is greater than the cost of the control method (Osteen, 1993). Economic thresholds for varroa mites have been developed to predict when treatments should be applied to prevent the mites from damaging colonies by reducing the adult and immature honey bee populations, colony survival, and honey production (e.g. Gatien and Currie, 1995; Delaplane and Hood, 1997, 1999; Strange and Sheppard, 2001; Gatien and Currie, 2003; Currie and Gatien, in review).

The economic threshold for varroa mites in honey bee colonies depends on several factors including the probability of reinfestation, the geographic region in which the bees are kept, and the time of year. In Nebraska, treatment is recommended if mites are detected at any level in the spring and when more than 2 mites per 100 bees are found in the fall (Ellis and Baxendale, 1996). In the southeastern region of the United States, treatment is recommended when more than 5 mites per 100 bees are detected in August or later or when 0.1 - 0.9 mites per 100 bees are detected in February (Delaplane and Hood, 1997, 1999). In Washington State, treatment is recommended if 1, 5, or 1 mites per 100 bees are found in April, August, or October, respectively (Strange and Sheppard, 2001). In Manitoba, Canada, mite levels of more than 2 mites per 100 bees in early spring result in lower worker populations and lost honey production (Gatien and Currie, 2003; Ostermann and Currie, 2004; Currie and Gatien, in prep.), but colonies can winter

successfully with mite levels of up to 10 mites per 100 bees in fall (Gatien and Currie, 2003).

The ability of colonies to tolerate moderate varroa mite infestations in the fall is critical if treatments in winter are to be feasible. If acaricide treatment can be delayed until winter, it might be possible to prevent winter loss of colonies while still bringing mite levels below the spring economic threshold for honey production in the subsequent summer. Strange and Sheppard (2001) examined the effect of acaricide treatment timing on honey bee colonies infested with varroa mites in Washington State. They found that, whether treating in April, August, October, April and October, or continuously, there is no difference in the size of the honey bee or varroa mite populations in November or the following April, indicating that treatment can be delayed from April to October as long as treatment occurs before winter (Strange and Sheppard, 2001). Calderone (2000) also treated colonies in October successfully in New York, allowing colonies to enter the following spring with very low mite populations. Gatien and Currie (2003) found that, in the prairie region of Canada, delaying treatment until winter is feasible when the mean abundance of varroa mites is below 10 mites per 100 bees in fall. Delaying treatment may not be possible in some geographic regions, particularly if there is a high rate of reinfestation from feral colonies. Delaplane and Hood (1997) found that delaying treatments from August to October in the southeastern United States causes a significant decline in the honey bee population the following December due to the effects of the increase in the mite population during the delay. In addition, Amdam et al. (2004) warn that delaying treatment may have an effect on the physiology of winter bees and Martin

(2001) states that varroa mites need to be controlled before winter bees are produced to reduce the establishment of honey bee viruses.

Using economic thresholds as a guide for treating colonies infested with varroa mites will prevent the overuse of acaricides while also preventing economic damage to colonies. Once the need for treatment is established, beekeepers must determine which method to use. Because of the establishment of varroa mite populations that are resistant to fluvalinate (e.g. Eischen, 1995; Lodesani et al., 1995; Milani, 1995; Colin et al., 1997; Elzen et al., 1998; Trouiller, 1998; Mozes-Koch et al., 2000; Floris et al., 2001) and coumaphos (e.g. Spreafico et al., 2001; Pettis, 2004), alternative methods are necessary for control. One such method, which is the focus of this dissertation, is the use of formic acid during winter.

FORMIC ACID

Formic acid is an inexpensive, readily available product that is a natural component of honey (Crane, 1979; Talpay, 1989; Liu et al., 1993; Bogdanov et al., 2002). Pure formic acid, CH_2O_2 , has a molecular weight of 46.03, a melting point of 8 °C, a boiling point of 101 °C, a specific gravity of 1.22, and a vapor pressure of 44.8 mm Hg at 20 °C (CCOHS, 2002). However, it is generally mixed with water to form a 65 % solution before it is applied as a fumigant within individual colonies outdoors in the spring or fall for control of varroa and tracheal mites, *Acarapis woodi* (Rennie). The physical properties of mixtures of aqueous formic acid at low temperatures are poorly understood. For the remainder of this dissertation, “formic acid” will be used to refer to

the aqueous solution and “pure formic acid” will be used to refer to the pure compound when not in solution.

Methods of use

Formic acid volatilizes readily in honey bee hives and acts as a fumigant. Various formulations for in-hive applications of formic acid have been investigated, but there are two major strategies that are followed; multiple pour-on applications with high in-hive concentrations for short time periods and slow-release treatments with low in-hive concentrations for long time periods. Formic acid is usually applied in the spring or fall for varroa mite control. Summer applications are avoided so that honey does not become contaminated (Stoya et al., 1986; Liu et al., 1993; Nelson et al., 1994; Bogdanov et al., 2002; PMRA, 1994). Label recommendations for these formulations indicate that treatments should be applied when daytime maximum temperatures are between 10 and 30 °C in spring or early fall (PMRA, 1994).

Pour-on treatments generally involve putting liquid formic acid onto absorbent materials, which are placed on the top bars or bottom board of the hive. The formic acid concentration in the air in hives treated in this way is high, but highly variable over time (Feldlaufer et al., 1997; Ostermann and Currie, 2004). On the day of application, concentrations of up to 222 ppm occur for a short time and then decrease quickly to a much lower concentration (Feldlaufer et al., 1997; Ostermann and Currie, 2004). The acid can evaporate completely from the absorbent materials within 2 days, requiring multiple applications if the fumigation is to continue for a duration equivalent to a brood cycle (Bracey and Fischer, 1989; Feldlaufer et al., 1997; Ostermann and Currie, 2004).

Pour-on formic acid treatments can be effective as a varroa mite control method. Treating colonies 4 - 6 times at 1 - 4 day intervals kills up to 99 % of varroa mites (Fries, 1989; Hoppe et al., 1989; Mutinelli et al., 1994; Clark, 1994, 1995; Eguaras et al., 1996). However, this treatment method can be expensive in terms of labor costs in commercial operations where there is a need to make additional trips to the apiary to apply multiple treatments and treatment efficacy is highly variable and dependent on climatic conditions (Gatien and Currie, 2003; Ostermann and Currie, 2004; Currie and Gatien, in review). In addition, working with liquid formic acid can be dangerous for the applicator because burns to skin and respiratory organs can occur (Thompson, 1992) and management that requires repeated handling of the acid increases the chance of accidental worker exposure.

Formic acid is also applied as a slow-release treatment using any one of several devices. Acid poured onto absorbent materials and placed in a plastic bag with many perforations or slits can be effective as a slow-release device (e.g. Krämer, 1980; Wachendörfer et al., 1983, 1985; Gatien and Currie, 1995; Parkman et al., 1999; Calderone and Nasr, 1999; Calderone, 1999, 2000; Elzen, 2003; Ostermann and Currie, 2004). Gel formulations also make effective slow-release devices (Clark, 1994; Feldlaufer et al., 1997; Parkman et al., 1999; Eguaras et al., 2001, 2003). Plastic containers with open vents in the lid or wicks also allow for slow release of formic acid vapors, but must be refilled repeatedly, diminishing their usefulness as labor-saving devices (Bracey and Fischer, 1989; Hood and McCreadie, 2001). The principle behind all of these slow-release products is to provide a relatively constant release of formic acid over several weeks of treatment.

During slow-release treatments, a solution of formic acid in water gradually volatilizes from a wick or pad over time, allowing a relatively constant or gradually decreasing weight loss during treatment (Feldlaufer et al., 1997; Calderone and Nasr, 1999; Parkman et al., 1999; Eguaras et al., 2001; Ostermann and Currie, 2004). Using a gel formulation, Feldlaufer et al. (1997) measured a maximum formic acid concentration of 50 ppm in hives and reported that the concentration did not drop below 10 ppm during the 21-day treatment period. Calderone and Nasr (1999) measured an average of 5 - 10 ppm in hives treated with an acid-soaked fiber board in a perforated plastic bag. Skinner et al. (2001) measured concentrations of 56 and 36 ppm during the first day of treatment with a gel and fiber board formulation, respectively. They found that, by day 13, the concentration in hives treated with the gel was 3 ppm, while with the fiber board the concentration was 15 ppm. Ostermann and Currie (2004) treated with a felt pad in a perforated plastic bag and found that the concentration in hives was 44 ppm on the day of application and 18 ppm 11 days later. Thus, using a slow-release formulation of formic acid, the relatively high concentrations found during rapid-release fumigation are avoided and treatment occurs over a longer period of time without increasing labor input.

The effectiveness of slow-release treatments against varroa mites is highly variable. Efficacy has ranged from 0 to 95 % varroa mite mortality (Clark, 1995; Feldlaufer et al., 1997; Calderone and Nasr, 1999; Calderone, 1999, 2000; Baxter et al., 2000; Eguaras et al., 2001, 2003). The variation in efficacy may be due to the effects of ambient temperature and colony size on the amount of formic acid volatilized into hive air (Feldlaufer et al., 1997; Skinner et al., 2001; Ostermann and Currie, 2004).

Volatilization of liquid from slow-release pads containing formic acid is positively related to ambient temperature (Feldlaufer et al., 1997; Parkman et al., 1999; Calderone and Nasr, 1999; Calderone, 1999, 2000; Hood and McCreadie, 2001). However, the effect of ambient temperature on in-hive concentrations is most dramatic early in the application period (Ostermann and Currie, 2004). Higher ambient temperatures and larger colony populations are associated with higher formic acid concentrations in hive air and faster rates of volatilization of formic acid (Ostermann and Currie, 2004). Therefore, the amount of formic acid to which the bees are exposed depends on both colony characteristics and daily weather patterns at the time of application (Ostermann and Currie, 2004). The effect of the variation in ambient temperature on formic acid efficacy is a major disadvantage of outdoor treatments.

Effect of treatment on honey bees

Both immature and adult honey bees can be negatively affected by formic acid fumigation. Ostermann and Currie (2004) found that, when varroa mite levels are below the economic threshold, slow-release formic acid treatment in the spring causes a decrease in worker brood production in some years that results in slower population build-up of honey bee colonies. In addition, formic acid treatment can decrease the production of drones (DeGuzman et al., 1999), a serious problem for queen breeders. Decreased brood production following formic acid treatment may be due to a decrease in brood care by nurse bees after prolonged formic acid treatment (Bolli et al., 1993) or to the direct effects of formic acid on larvae (Gregorc et al., 2004). Adult worker bees exposed to either slow-release or rapid-release treatments can also have greater mortality rates than untreated bees (Nelson et al., 1994; Elzen, 2003). In a laboratory study,

Lindberg et al. (2000) found that formic acid is not highly selective dividing the LD₅₀ of honey bees by LD₅₀ varroa mites to get a selectivity ratio of 2.1:1.

Formic acid fumigation does not always negatively affect honey bees. Using a slow-release treatment method in fall, Calderone (2000) did not see an effect of treatment on colony survival over winter or on the quantity of bees, brood, pollen or honey in the subsequent spring. Using a slow-release treatment method in spring, Ostermann and Currie (2004) found that treatment of colonies containing varroa mites resulted in greater brood production and a larger bee population than in untreated colonies. Nelson et al. (1994) and Westcott and Winston (1999) found that formic acid treatment with either a slow-release or pour-on treatment in spring did not affect sealed brood area or honey production following treatment. Others, using a variety of formic acid treatments, have also reported no negative effect of formic acid fumigation on bees (Garg et al., 1984; Hoppe et al., 1989; Sharma and Raj, 1999; Skinner et al., 2001; Sharma et al., 1994, 2003; Gatien and Currie, 2003; Currie and Gatien, in review).

Queen loss during formic acid treatment has been reported by some researchers, but was not seen by others. No queens were lost in several studies using slow-release treatments (Bracey and Fischer, 1989; Hoppe et al., 1989; Sharma et al., 1994, 2003; Ostermann and Currie, 2004; Currie and Gatien, in review). However, queen losses of 5-10 % were reported when rapid-release treatments were used (VonPoseern, 1988; Fries, 1989) and losses of 20 % were reported when slow-release treatments were used (Wachendörfer et al., 1983; Elzen, 2003). The cause of queen loss was not determined in any of the studies, but the potential for queen loss is an important aspect of this treatment method that must be considered.

It has been suggested that queen loss during fumigation is associated with high ambient temperatures (VonPosern, 1988; PMRA, 1994). However, there is little evidence to support this, as the details of the conditions when queen loss has occurred have not been reported. Few researchers have reported ambient temperatures during experiments and those that have provide little support for this hypothesis. For example, Elzen (2003) observed 20 % queen loss when using a gel formulation of formic acid when temperatures averaged 15.5 °C in the first 6 days of fumigation and 24.1 °C thereafter, ranging from 10 - 40 °C. These temperatures are well within the range considered acceptable for fumigation (PMRA, 1994). Because formic acid concentration in hive air during fumigation is correlated with ambient temperature (Calderone and Nasr, 1999; Ostermann and Currie, 2004), high ambient temperature could increase queen loss through increased exposure of queens to a high concentration of formic acid. However, acid concentration in fumigated hives was not measured in any studies reporting queen loss, so a link between acid concentration and queen loss cannot be made. More work needs to be done to determine the conditions that cause queen loss during formic acid fumigation.

RATIONALE OF THIS STUDY

A possible alternative method for treating varroa mite-infested honey bee colonies with formic acid is fumigation indoors in winter. Treating in winter is advantageous for several reasons. During the early winter months in northern regions, little or no honey bee brood is present in the hive (Harris, 1980; Möbus, 1998). Therefore, varroa mite reproduction is greatly reduced and most of the mites would be found on adult honey

bees (DeJong, 1990) where they are more likely to be exposed to acaricide treatments. In addition, treatment indoors would allow for control of ambient conditions. Because formic acid volatilization varies with ambient temperature (Feldlaufer et al., 1997; Parkman et al., 1999; Calderone and Nasr, 1999; Calderone, 1999, 2000; Hood and McCreddie, 2001; Ostermann and Currie, 2004), treatment concentrations can change over time when formic acid is applied outdoors. Treating indoors might allow more stable and predictable treatment concentrations, which could decrease the variability of treatment efficacy. Lastly, fumigating entire rooms containing many colonies instead of individual hives will save labor costs involved in treatment. The amount of chemical handling will be decreased as well, reducing the danger of injury to the applicator through accidental spills. Overall, formic acid fumigation indoors in winter is a promising varroa mite control technique with many advantages over its use outdoors.

This study is an examination of the use of formic acid as a fumigant indoors in winter. The main objectives are to apply the concepts related to the principles of fumigation as an insect control measure to formic acid fumigation of honey bees, to determine the feasibility of this method as a pest control technique, and to determine the treatment regime that causes the maximum varroa mite mortality while causing the minimum effect on honey bees.

CHAPTER 1. THE EFFECTS OF TEMPERATURE AND CONCENTRATION OF
FORMIC ACID ON TREATMENT EFFICACY AGAINST THE HONEY BEE, *APIS
MELLIFERA* L. (HYMENOPTERA: APIDAE), PARASITE *VARROA DESTRUCTOR*
ANDERSON AND TRUEMAN (ACARI: VARROIDAE)

ABSTRACT

To decrease the variability of formic acid treatments against the honey bee parasite the varroa mite, *Varroa destructor* Anderson and Trueman, it is necessary to determine the concentration-time combination that best controls mites without harming bees. The concentration x time (CT) product is a valuable tool for studying fumigants and how they might perform under various environmental conditions. This laboratory study is an assessment of the efficacy of formic acid against the varroa mite under a range of formic acid concentrations and temperatures. The objectives are 1) to determine the effect of temperature and concentration of formic acid on worker honey bee and varroa mite survival, 2) to determine the CT₅₀ products for both honey bees and varroa mites and 3) to determine the best temperature and concentration to optimize selectivity of formic acid treatment for control of varroa mites. Worker honey bees and varroa mites were fumigated at 0, 0.01, 0.02, 0.04, 0.08, and 0.16 mg/L at 5, 15, 25, and 35°C for 12 days. Mite and bee mortality were assessed at regular intervals.

Both mite and bee survival were affected by formic acid concentration. Concentrations of 0.08 and 0.16 mg/L were effective at killing mites at all temperatures tested above 5°C. There was a significant interaction between temperature, concentration, and species for the CT₅₀ product. The difference between the CT₅₀

product of bees and mites was significant at only a few temperature-concentration combinations. CT product values showed that at most temperatures the greatest fumigation efficiency occurred at lower concentrations of formic acid. However, the best fumigation efficiency and selectivity combination for treatments occurred at a concentration of 0.16 mg/L when the temperature was 35°C.

INTRODUCTION

The varroa mite, *Varroa destructor* (Anderson and Trueman, 2000), is an introduced ectoparasitic mite that feeds on the hemolymph of adult and immature honey bees (*Apis mellifera* L.). This pest has cost the beekeeping industry millions of dollars due to loss of honey yield and mortality of honey bee colonies. Effective mite control methods are necessary to prevent severe losses.

The contact poison, fluvalinate-tau, is generally a highly effective control for varroa mites. However, varroa mite resistance to this method of control is already a problem (e.g. Eischen, 1995; Lodesani et al., 1995; Elzen et al., 1998). Formic acid fumigation is an alternative control measure that has the advantages that no documented resistance to the chemical has occurred (VonPoseern, 1988), it is inexpensive (Liu, 1991), does not leave residues above natural levels already in honey (Stoya et al., 1986; VonPoseern, 1988; Liu et al., 1993; Bogdanov et al., 1999), and does not usually harm adult worker honey bees (e. g. Koeniger and Rau, 1980; Garg et al., 1984; Lupo and Gerling, 1990; Westcott and Winston, 1999). However, the efficacy of this compound varies considerably under current treatment methods. Even identical treatments have shown highly variable results (Charriere et al., 1992).

There are a number of factors known to affect treatment efficacy. The distance from the site of formic acid volatilization to the comb containing bees and the placement of the acid in the hive above or below the brood chamber both affect treatment efficacy (Fries, 1989). Factors such as the amount of brood in the hive (Eischen, 1998), time of year (Maul et al., 1980), and ambient temperature (e.g. VonPoseern, 1988; Feldlaufer et

al., 1997; Eischen, 1998; Calderone and Nasr, 1999; Skinner et al., 2001) have also been suggested to affect treatment efficacy.

The actual concentration of formic acid in the air in the honey bee hive during treatment varies with temperature and application method. To date, most studies have not measured the actual concentration to which the honey bees are exposed, but instead report treatment doses as weights or volumes of acid evaporated into a hive over a given time period (e. g. Bracey and Fischer, 1989; Fries, 1989; Calderone, 1999; Baxter et al., 2000). This method of measurement does not quantify the actual concentration of formic acid in air or allow calculation of the amount of time the mites are exposed to the treatment. Some studies have quantified the amount of formic acid in hive air, but have not identified the concentration-time combinations required to kill mites under different environmental conditions (Charriere et al., 1992; Feldlaufer et al., 1997; Skinner et al., 2001). To improve the reliability and efficacy of varroa mite fumigation with formic acid, we need to first determine the combination of concentration (measured in ppm) and exposure time necessary to control mites at various temperatures.

The combination of acid concentration and exposure time can be reported as the concentration x time (CT) product (Monro, 1969). This value is determined by experimentally assessing the amount of fumigant (measured in mg/L in air) and length of time necessary to kill x % of the test individuals (CT_x) (Bond, 1984). Generally, as the concentration increases, the amount of time necessary to kill a pest decreases, yielding a relatively stable and predictable CT product that can be used in a variety of practical situations (Harein and Krause, 1964; Estes, 1965). Temperature can affect the CT product, so various concentrations of the fumigant must be tested under a range of

temperatures. The CT product value at different temperatures can then be used to extrapolate the amount of time and concentration necessary to control a pest under practical conditions. If these values could be established for varroa mites and honey bees, fumigation systems for controlling mites could be designed to ensure that the desired CT product is realized under a variety of environmental conditions.

In this study, we assessed the efficiency of formic acid in a controlled laboratory environment under a range of temperatures and formic acid concentrations. The objectives of this research were 1) to determine the effect of temperature and concentration of formic acid on worker honey bee and varroa mite survival, 2) to determine the CT_{50} products for both honey bees and varroa mites and 3) to determine the selectivity of formic acid treatment for control of varroa mites.

METHODS AND MATERIALS

Experimental Setup

Bioassay cages had outer dimensions of 20.5 x 8.0 x 11.2 cm. Within each cage, bees were confined to a 15.8 x 8.8 x 5.0 cm space. The two sides, back and the top of the cage were solid wood, the front was a fine mesh screen (14 squares per cm) and the removable floor was 1.7-squares-per-cm hardware cloth. Below the floor was a drawer that could be removed to count fallen mites and bees without allowing live bees to escape. The cage top had two 3.5 cm diameter holes into which 50 mL centrifuge feeder tubes were placed. The lid of each centrifuge tube had a 2 cm diameter hole cut into it that was covered with a fine mesh (25 squares per cm). One feeder tube contained

distilled water and the other contained 67% sucrose in distilled water as measured by a refractometer. Each tube was refilled when necessary.

Mixed age worker honey bees were collected from colonies with no or low mite loads, anesthetized with CO₂, counted, and placed in the cages in groups of 300. Each cage was infested with 30 live varroa mites. Mites were collected by a water wash technique (Currie, in prep.) as follows. Bees from highly infested colonies were anesthetized with CO₂ and shaken into a 0.5 L plastic container with a hardware cloth (1.7 squares per cm) lid. The container was then placed in a bucket of warm, distilled water and allowed to soak for five minutes. After soaking, the containers were vigorously shaken in the water to dislodge mites. The water in the bucket was then poured through a fine mesh cloth (25 squares per cm) that trapped the mites. The mites were then picked up with the tip of a soft paint brush and placed on a paper towel. Mites quickly recovered and were introduced into bioassay cages.

The cages were then placed in temperature-controlled chambers and held under constant darkness at 30°C for 24 hours. On day 0, cages were randomly assigned to each treatment combination. Bioassay cages were placed in a flow-through fumigation system for the duration of the 12-day treatment. Humidified air was blown through a clear vinyl tube (0.63 cm inner diameter, Anderson Barrows Metals Corporation, Palmdale, CA) at a rate of 400 mL/min into a 1L mason jar containing 300 mL of a known concentration of formic acid in distilled water. The air was bubbled through the liquid and moved through a tube that led to the bioassay cage. The cage was confined in a closed 45 x 55 cm Mylar bag (Reckitt and Colman Canada Inc., Toronto, Ontario) to prevent leakage of any formic

acid vapor into the chamber. Air coming from the bag was exhausted through an additional tube into a fume hood.

Bioassay cages were treated with six concentrations of formic acid in air; 0.00, 0.01, 0.02, 0.04, 0.08, and 0.16 mg/L (0, 5, 10, 20, 40 and 80 parts per million). Each concentration was tested at each of four temperatures; 5, 15, 25, and 35°C. Thus, there was a total of 24 treatment combinations. The experiment was replicated three times. For each replicate, all of the worker bees were taken from a single colony and a different colony was used for each replicate. Varroa mites were collected from a number of colonies and assigned to each cage without knowledge of their origin. Treatments were randomly assigned to each cage. Each day, dead bees and mites were removed from the test cages and counted. Fumigation ended after 12 days and any remaining live bees and mites were anesthetized with CO₂ and counted.

Formic Acid Concentration

Mason jars containing different concentrations of formic acid in water were placed within the system to alter the amount of formic acid in air. Prior to the onset of the experiment, the concentration of formic acid in the air within the system was measured using an air sampling system. A gas detector pump (Accuro 9022003, National Dräger, Pittsburgh, Pennsylvania) was used to suck air through a Dräger tube (no. 6722101) as per the manufacturer's instructions. A standard curve for each temperature was created to determine the percent of acid in the jar that gave a desired formic acid concentration in air. Acid concentration in air was not monitored during the fumigation period.

Data Analysis

Mite and worker bee survival at each concentration of formic acid was compared to survival of the control within each temperature treatment combination (LIFETEST, SAS Institute Inc., 1999). A CT_{50} product, the combination of concentration and time of exposure that caused 50% mortality in mites or worker bees, was calculated for each cage after correcting for mortality in the control using Abbott's formula (Abbott, 1925). The CT_{50} product value data were analyzed using a split-plot design with formic acid concentration, temperature, and replicate as main plot factors and species within cage as a subplot factor. Comparisons of the CT_{50} products between bees and mites within each temperature and concentration and the effects of concentration and temperature on the CT_{50} product of each species were analyzed using orthogonal contrasts with Bonferroni-corrected alpha values.

RESULTS

Survival

Mite survival was affected by formic acid concentration, but the survival response to different formic acid concentrations varied with temperature. At 5°C formic acid treatment reduced mite survival only at a concentration of 0.16 mg/L and only at early survival times as indicated by the Wilcoxon test (Fig. 1.1a). The two highest concentrations were effective in killing mites at all other temperatures (Fig. 1.1b-d). However, at 25°C, all concentrations tested (0.01 mg/L or higher) reduced mite survival relative to controls (Fig. 1.1c).

At all temperatures, honey bee worker survival relative to controls was affected by formic acid treatment (Fig. 1.2). However, the effect of formic acid was not always negative. Bee survival was slightly higher than in untreated controls at concentrations of 0.02 and 0.04 mg/L at 5°C (Fig. 1.2a). Bees showed reduced survival relative to controls for most formic acid concentrations at temperatures higher than 5°C (Fig. 1.2b-d). At 15°C, treatments of 0.04 mg/L or higher reduced bee survival relative to controls (Fig. 1.2b) and at 25 and 35°C, all concentrations reduced bee survival relative to controls (Fig. 1.2c, d).

CT Products

The CT products of bees and mites at various temperature and concentration combinations are shown in Fig. 1.3. There was a significant interaction between temperature, concentration, and species ($F = 4.25$; $df = 12, 40$; $P = 0.0003$) for the CT₅₀ product. At 35°C there was a significant difference between the CT₅₀ products of bees and mites for the concentrations of 0.08 mg/L and 0.16 mg/L (0.08 mg/L; $F = 23.56$, $df = 1, 40$, $P < 0.025$; 0.16 mg/L; $F = 94.24$, $df = 1, 40$, $P < 0.025$; Fig. 1.3d). At 15°C the difference between the CT₅₀ product of bees and mites was significant only for a concentration of 0.08 mg/L ($F = 16.36$; $df = 1, 40$; $P < 0.05$; Fig. 1.3b).

The CT₅₀ product of both bees and mites was significantly affected by temperature at formic acid concentrations of 0.04, 0.08, and 0.16 mg/L (bees, 0.04 mg/L; $F = 10.80$, $df = 3, 40$, $P < 0.025$; bees, 0.08 mg/L; $F = 65.00$, $df = 3, 40$, $P < 0.025$; bees, 0.16 mg/L; $F = 32.58$, $df = 1, 40$; $P < 0.025$; mites, 0.04 mg/L; $F = 17.56$, $df = 3, 40$, $P < 0.025$; mites, 0.08 mg/L; $F = 51.06$, $df = 3, 40$, $P < 0.025$; mites, 0.16 mg/L; $F = 155.23$, $df = 3, 40$, $P < 0.025$; Fig. 1.3). The relative response of the CT₅₀ product to temperature

was not consistent across all concentrations. For both bees and mites at the concentration of 0.16 mg/L, the CT_{50} products decreased as temperature increased. On the other hand, at a concentration of 0.08 mg/L, the CT_{50} products increased between 5 and 15°C, decreased between 15 and 25°C, and increased again between 25 and 35°C.

Formic acid concentration also significantly affected the CT_{50} product of bees and mites at all temperatures (bees, 5°C; $F = 128.50$, $df = 4, 40$, $P < 0.025$; bees, 15°C; $F = 110.04$, $df = 4, 40$, $P < 0.025$; bees, 25°C; $F = 67.04$, $df = 4, 40$, $P < 0.025$; bees, 35°C; $F = 36.79$, $df = 4, 40$, $P < 0.025$; mites, 5°C; $F = 148.01$, $df = 4, 40$, $P < 0.025$; mites, 15°C; $F = 74.15$, $df = 4, 40$, $P < 0.025$; mites, 25°C; $F = 62.57$, $df = 4, 40$, $P < 0.025$; mites, 35°C; $F = 10.05$, $df = 4, 40$, $P < 0.025$; Fig. 1.3). At 15 and 35°C, the CT_{50} product for both bees and mites increased from 0.01 to 0.08 mg/L and decreased from 0.08 to 0.16 mg/L. In contrast, at 5 and 15°C, the CT_{50} product increased or stayed the same from 0.01 to 0.16 mg/L.

DISCUSSION

The two highest concentrations of formic acid tested, 0.08 and 0.16 mg/L, were effective at reducing mite survival at all temperatures tested above 5°C. The effect of formic acid on varroa mites and worker bees varied with both temperature during fumigation and concentration of formic acid in the air. There were differences in the concentration-temperature combinations required to kill mites and bees, but only a few temperature-concentration combinations were more effective at killing mites than bees.

Concentrations of 0.08 and 0.16 mg/L of formic acid were consistently effective against varroa mites in this study at temperatures above 5°C. These concentrations are

similar to those measured in hive air in field studies of formic acid fumigation. Studies of slow release formulations have shown that formic acid levels within the hive range from 10 - 64 ppm (0.02 – 0.13 mg/L) (Feldlaufer et al., 1997; Skinner et al., 2001; Ostermann and Currie, 2004). In-hive concentrations of formic acid for pour-on formulations range from 19-400 ppm (0.04 – 0.22 mg/L) on the day of application and decrease to an average of 18 ppm (0.04 mg/L) after 11 days (Feldlaufer et al., 1997; Imdorf et al., 1999; Ostermann and Currie, 2004)

In full size colonies with concentrations of formic acid similar to those used in our study, significant varroa mite mortality can be achieved without killing large numbers of adult worker bees (Skinner et al., 2001). In our study, worker longevity was reduced by formic acid concentrations similar to those found in field studies. This may be because the bees were confined to a cage and were not able to move out of the acid-laden air or move to a different temperature as they would in colonies that are treated outdoors. In addition, our caged bees were queenless and this would likely reduce the survival of bees in all treatments, including the control. The presence of a queen in the bioassay cages might increase survival of workers relative to varroa mites, and thus provide a better estimate of field conditions, but this would significantly increase the costs associated with the bioassay.

Lindberg et al. (2000) applied formic acid to varroa mites and honey bees in Petri dishes at 30°C and showed that formic acid is not a highly selective mite treatment. They found that the selectivity ratio of the LD₅₀ of workers divided by the LD₅₀ of mites was only 2.1. Using the CT₅₀ values from this study, a higher selectivity ratio of 5.0 was seen at 35°C when 0.16 mg/L formic acid was applied. However, at lower concentrations and

temperatures, the selectivity ratios were very small, the lowest being 0.9 when 0.02 mg/L formic acid was applied at 25°C. Thus, our results show that consideration of temperature is critical in assessing selectivity of formic acid against bees and mites. At high concentrations tested at 15 and 35°C, the difference between the CT_{50} of bees and the CT_{50} of mites was significant, whereas there were no significant differences in selectivity at other temperature-concentration combinations.

Because of the social nature of bees, the incubator temperature is not necessarily equivalent to the temperature to which the bees were exposed. Honey bees cluster together at low temperatures ($<18^{\circ}\text{C}$), creating a temperature in the cluster that is higher than the ambient temperature (Simpson, 1961). The bees in our cages that were held at 15 and 5°C also clustered and the higher temperatures in the cluster may explain why the formic treatment was more selective at 15°C than at 25°C , a temperature at which the bees did not cluster. The relatively high selectivity at high concentrations suggests that short-term high-concentration treatments have the potential to be most effective in killing varroa mites while minimizing the effect on bees when application is carried out at brood nest temperatures.

In our experiment, the caged bees kept at 5°C often died early in the experiment. This was likely because the small clusters of bees we used could not maintain their temperature and was not caused by a treatment effect. To properly assess the efficacy of formic acid and other fumigants under cooler ambient conditions larger clusters of bees would be required.

Studies have shown that toxicity of fumigants increases with temperature (e.g. Estes, 1965; Batth, 1971; Bond, 1975). In this study, temperature of fumigation had

similar effects. Overall, the CT_{50} products of both bees and mites tended to decrease with increasing temperature, but the CT_{50} products at higher concentrations of formic acid were affected more strongly by temperature than lower concentrations. In addition, the CT_{50} product of the varroa mites decreased more strongly than that of the bees at higher temperatures. Fumigants often perform better at higher temperatures because the metabolic rate of the target species is greater (Monro, 1969) and/or the insect is more likely to be exposed to the treatment. It is not known whether the increased effectiveness of formic acid against varroa mites at higher temperatures that we observed is due to increased metabolic activity of mites, greater movement of the mite on the host, or both.

For many organisms, the CT product remains constant for given doses of a fumigant (Estes, 1965). However, Estes (1965) and Harein and Krause (1964) found that for some species of grain beetles, CT_{50} and CT_{95} products increased as the length of exposure to the fumigant increased, indicating decreased fumigation efficiency at longer exposure times. Decreased fumigation efficiency at higher concentrations is often associated with a form of protective stupefaction that results in organisms slowing down their respiration when exposed to a sudden high concentration of a fumigant (Estes, 1965; Bond et al., 1969). Our results show that the CT product for both bees and mites tended to increase with formic acid concentration, which implies a lower efficiency at higher concentrations for most temperature-concentration combinations. Some form of protective stupefaction may have occurred when bees and mites were exposed to high concentrations of formic acid in some treatment combinations, but we do not have any direct evidence of this.

The CT product is a reliable value that can be used to standardize treatments with fumigants. This study is the first to determine a CT product for formic acid fumigation of varroa mites and honey bees at a range of temperatures. Our results showed that the CT product changed with concentration and temperature and this may help explain why treatment efficacy is so variable in the field. Our study showed that the most effective concentrations for killing mites with minimal worker bee mortality were 0.08 and 0.16 mg/L. Fumigation efficiency against varroa mites was greater at lower concentrations, but the differences in selectivity of treatment between the parasite and its host were much smaller. The best fumigation efficiency and selectivity combination for treatments occurred at a concentration of 0.16 mg/L when the temperature was 35°C. This suggests that formic acid would be most effective as a fumigant against varroa mites at brood nest temperatures when short-term high concentration treatments are applied.

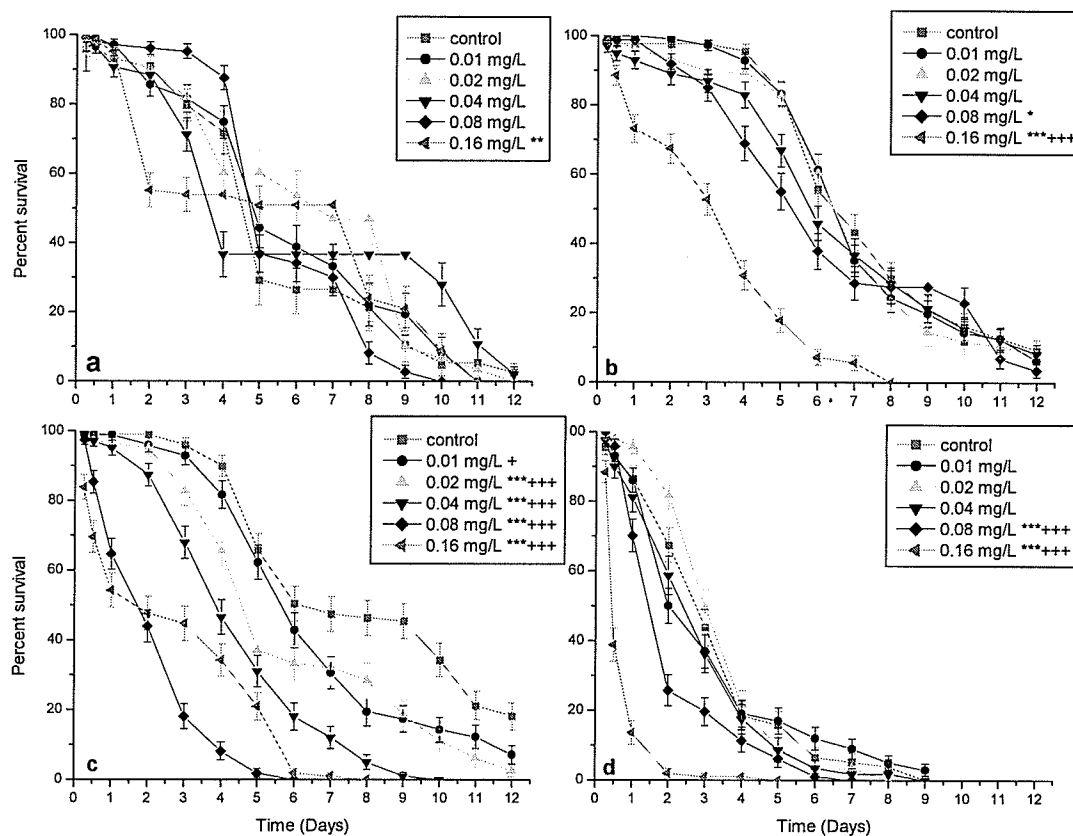


Figure 1.1. Varroa mite survival at 5°C (a), 15°C (b), 25°C (c), and 35°C (d) over the duration of the experiment. N for each point is approximately 90 mites.

Significant differences between a treatment and the control are denoted as

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ according to the Wilcoxon test and + $P < 0.05$, ++ $P < 0.01$, and +++ $P < 0.001$ according to the Log-Rank test of the LIFETEST procedure.

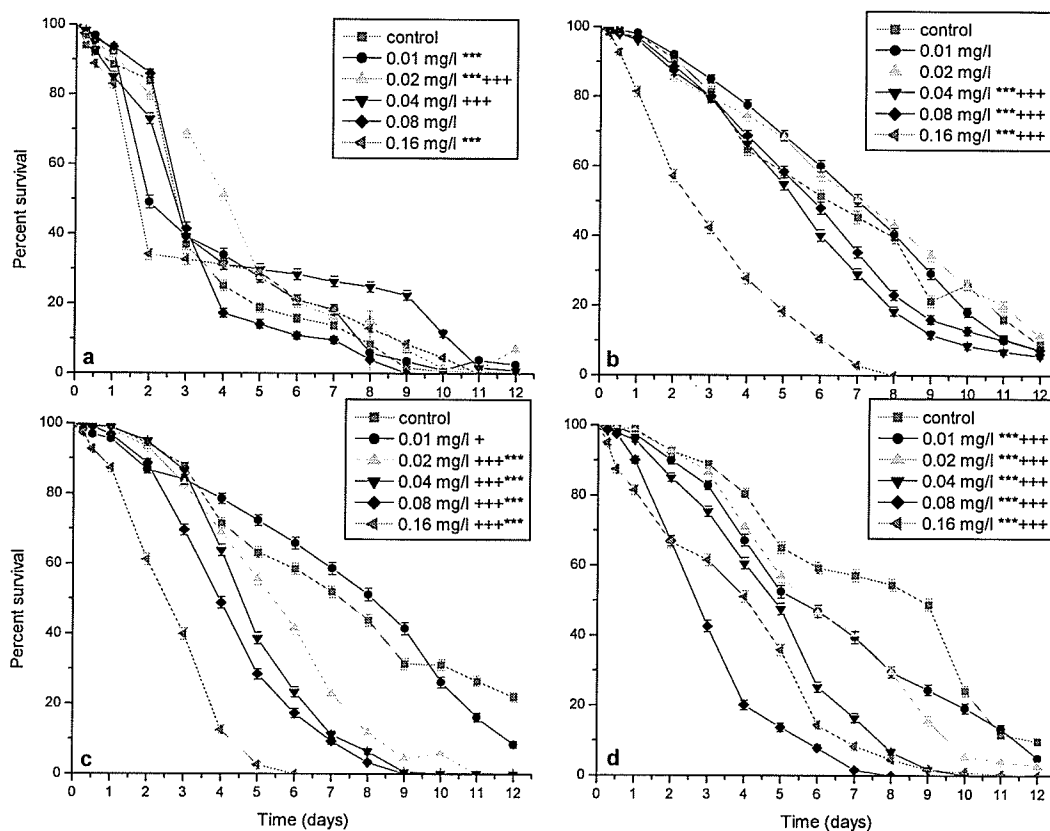


Figure 1.2. Worker bee survival at 5°C (a), 15°C (b), 25°C (c), and 35°C (d) over the duration of the experiment. N for each point is approximately 900 bees.

Significant differences between a treatment and the control are denoted as

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ according to the Wilcoxon test and + $P < 0.05$,

++ $P < 0.01$, and +++ $P < 0.001$ according to the Log-Rank test of the LIFETEST procedure.

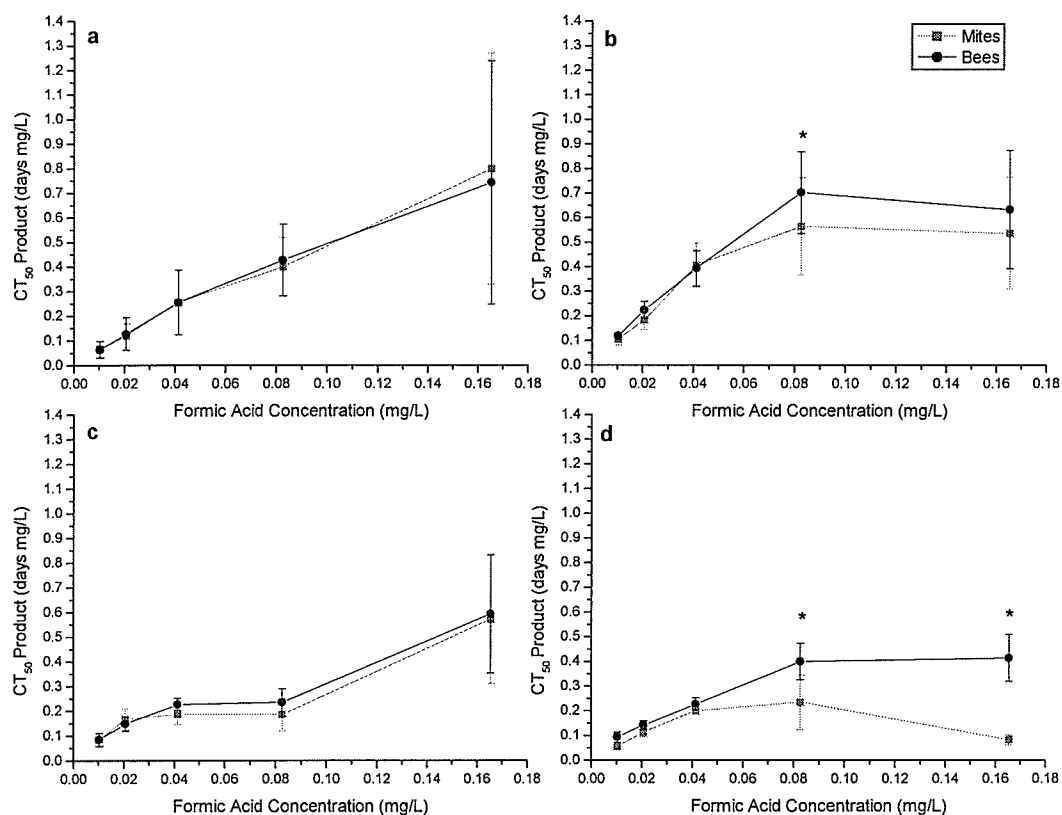


Figure 1.3. CT₅₀ products for bees and mites at 5°C (a), 15°C (b), 25°C (c), and 35°C (d).

Points are means \pm SD for 3 replicates. Values are corrected for mortality in the control using Abbott's formula. Significant differences ($P < 0.01$ in all cases) between the CT product of bees and mites are denoted by an asterisk (*).

CHAPTER 2. INDOOR WINTER FUMIGATION OF *APIS MELLIFERA* L.
(HYMENOPTERA: APIDAE) COLONIES INFESTED WITH *VARROA DESTRUCTOR*
ANDERSON AND TRUEMAN (ACARI: VARROIDAE) WITH FORMIC ACID IS A
POTENTIAL CONTROL ALTERNATIVE IN NORTHERN CLIMATES

ABSTRACT

Formic acid treatment for the control of the ectoparasitic varroa mite, *Varroa destructor* Anderson and Trueman, infesting honey bee, *Apis mellifera* L., colonies is usually carried out as an in-hive outdoor treatment. This study examined the use of formic acid on wintered colonies kept indoors at 5 °C from 24 November 1999 to 24 March 2000. Colonies were placed in small treatment rooms that were not treated (control) or fumigated at three different concentrations of formic acid: low (mean 11.9 ± 1.2 ppm), medium (mean 25.8 ± 1.4 ppm), or high (mean 41.2 ± 3.3 ppm), for 48 hours on 22-24 January 2000. Queen bee, worker bee, and varroa mite mortality were monitored throughout the winter, and tracheal mite, *Acarapis woodi* (Rennie), prevalence and mean abundance of nosema, *Nosema apis* Zander, spores were assessed.

This study revealed that formic acid fumigation of indoor-wintered honey bees is feasible and effective. The highest concentration significantly reduced the mean abundance of varroa mites and nosema spores without increasing worker bee mortality. Tracheal mite prevalence did not change significantly at any concentration, although we did not measure mortality directly. The highest concentration treatment killed 33.3 % of queens compared to 4.8 % loss in the control. Repeated fumigation periods at high concentrations or extended fumigation at low concentrations may increase the efficacy of

this treatment method and should be tested in future studies. An understanding of the cause of queen loss and methods to prevent it must be developed for this method to be generally accepted.

INTRODUCTION

During early winter in northern regions, little or no honey bee, *Apis mellifera* L., brood is present in the hive (Harris, 1980; Möbus, 1998). At this time of year, the ectoparasitic mite, *Varroa destructor* Anderson and Trueman, which reproduces in brood cells with developing honey bees, also halts its reproduction (DeJong, 1990). In broodless periods, mites rely on adult honey bees for food (DeJong, 1990) and are, thus, more likely to be exposed to acaricide treatments. Therefore, winter may be an ideal time to treat varroa-infested colonies if a treatment method can be applied without harming the bees. In the prairie region of Canada where reinfestation from feral colonies is limited, a single treatment during this yearly gap in mite reproduction may provide sufficient control of varroa mites for the entire year.

Honey bee colonies can be overwintered indoors or outdoors. In northern climates, outdoor wintering involves placing hives in a sheltered location and wrapping them with insulation that serves to slow down temperature changes, conserve heat, and decrease moisture within the hives (Gruszka, 1998). An alternative method that is used by many commercial beekeepers in Canada and by a limited number of beekeepers in the United States is to winter bees indoors. In indoor wintering, hives are typically moved for the winter into a building that is maintained at 5 °C in darkness with proper air circulation and ventilation (Gruszka, 1998). Under these controlled environmental conditions, honey bee colonies survive very well even in areas with the harshest of winters. Commercial beekeepers typically house 1,000 to 2,000 colonies in a single building, and all of these colonies could be treated for diseases at the same time in a single application if an effective fumigant were available.

One fumigant that may be suited for treatment in wintering buildings is formic acid. Indoor formic acid fumigation is advantageous because there is a high degree of control over ambient environmental conditions to which colonies are exposed relative to outdoor applications. With a wintering building, the ambient temperature can be maintained within a narrow range. During field use, volatilization of formic acid is affected by a number of environmental variables, which in turn causes concentrations of formic acid in the hive to fluctuate widely (Charriere et al., 1992; Feldlaufer et al., 1997; Skinner et al., 2001; Ostermann, 2002). Variation in the concentration of formic acid in hive air explains some of the high variation in efficacy of formic acid against varroa mites (Charriere et al., 1992; Ostermann, 2002).

Laboratory studies have shown that temperature also has a direct effect on the efficacy of formic acid against varroa mites and that the acid is less effective in killing varroa mites at lower temperatures (Underwood and Currie, 2003). Label recommendations for formic acid in-hive treatments in Canada suggest that formic acid is ineffective when the ambient temperature drops below 10 °C. Although this suggests that fumigation at low temperatures might not be feasible, the temperature in the winter cluster ranges from 10 to 30 °C (Simpson, 1961). At these temperatures, concentrations of 40 and 80 ppm are effective in controlling varroa mites (Underwood and Currie, 2003). Therefore, it should be possible to fumigate colonies in a wintering facility providing that the appropriate concentrations of formic acid can be volatilized and delivered to colonies at low temperatures.

The objective of this study was to assess the feasibility of using formic acid fumigation to control varroa mites in a wintering building. A number of formic acid

concentrations were tested on indoor-wintered honey bee colonies infested with varroa mites. The effects of different concentrations of formic acid on varroa mites; nosema disease, *Nosema apis* Zander; tracheal mites, *Acarapis woodi* (Rennie); worker bees; queen bees; colony survival; and colony weight were measured.

MATERIALS AND METHODS

Time line of events

Eighty-four honey bee colonies were obtained from southern Manitoba beekeepers in early November 1999 and brought to the University of Manitoba apiary in Winnipeg, Manitoba, Canada. On 24 November 1999, colonies were assessed to determine initial pest abundance means and colony characteristics and were moved into a large holding room in the wintering building on the University of Manitoba campus. On 12 January 2000, hives were moved into treatment rooms within the wintering building and set up for treatment. Three treatment rooms were fumigated with formic acid for 48 hours from 22 to 24 January 2000, while the fourth went untreated. All hives remained in the building until 23 March 2000 when they were moved outside to the University of Manitoba apiary. Final pest abundance means and colony characteristics were measured on 24 March 2000.

Pre-treatment sampling

Each colony was contained in one full-depth Langstroth hive body containing nine or 10 frames. Lid type varied with beekeeper. On 24 November 1999, all 84 colonies were sampled to assess worker cluster size, mean abundance of varroa mites and nosema spores, tracheal mite prevalence, and hive weight. Cluster size was ranked (at a

temperature between -9 and -5 °C) and given a score from 1 to 3 using the following criteria: a score of 1 was given for a cluster covering three or fewer frames, a score of 2 was given for a cluster covering four to six frames, and a score of 3 was given for a cluster covering seven or more frames. A sample of 200-300 adult worker bees was collected from each colony to estimate parasite and disease levels. Mean abundance (Bush et al., 1997) of varroa mites on adult workers was estimated by an alcohol shake method as follows. Workers were placed in a box (26.5 by 15.0 by 8.5 cm) with a metal screen floor (2.3 squares per cm) raised 1 cm from the bottom of the box. The bees and box were placed in a larger closed plastic container (30.0 by 9.0 by 8.5 cm, Rubbermaid, Wooster, OH) containing 1,500 mL of 70 % ethanol. The entire container was shaken on a mechanical shaker (Lab-line Instruments, Inc., Melrose Park, IL) for 10 min at 150 rpm. The number of mites that dropped through the screen was divided by the number of bees that were shaken and multiplied by 100 to estimate the mean abundance of varroa mites in each colony (no. mites per 100 bees).

After each sample was shaken, 100 bees were taken for an assessment of mean abundance of nosema spores and tracheal mite prevalence. Mean abundance of nosema spores was assessed according to the methods of Cantwell (1970). Tracheal mite prevalence was assessed according to the methods of Delfinado-Baker (1984). After the pre-treatment samples were taken, the hives were weighed and moved into a large storage room in the wintering building at the University of Manitoba and maintained there under constant darkness at 5°C.

Hive setup

On 12 January 2000, 21 hives were moved into each of four small treatment rooms (3.0 by 1.7 by 2.7 m; Fig. 2.1) within the wintering building. Colonies were sorted based on pre-treatment mean varroa abundance and colony weight and then divided into 21 blocks of four hives each. One hive from each of the blocks was randomly assigned to each treatment room.

Once the hives were in place in the treatment rooms, hives in positions 1-5 (Fig. 2.1) were fitted with temperature probes (HOBO[®] data logger, Onset Computer Corporation, Bourne, MA). In all treatment rooms but the control room, hives 1-3 were also fitted with 4.3-mm inner diameter polyethylene air-sampling tubes (Anderson Barrows Metals Corporation, Palmdale, CA). Data loggers and air-sampling tubes were attached directly to the comb with a thin wire and located at the edge and center of the honey bee cluster. Each air-sampling tube ran from the point of measurement to a site outside the room where it was sealed with a rubber sleeve stopper (5.2-6.7-mm plug diameter, 03-215-5, Fisher Limited, Ottawa, Ontario, Canada). Thus, air samples could be collected during fumigation without entering the room.

A piece of white poster board (36 by 56 cm) was placed on the bottom board of each hive to allow fallen varroa mites and bees to be collected throughout the experiment. Additional dead bees were collected outside of the hive entrance in a dead bee trap (aluminum, three-sided box, 47 by 19 by 8 cm; Fig. 2.1g). All bees and mites falling onto the white board or into the aluminum pan were collected from all 84 hives at six points throughout the winter. The first collection consisted of a 2-day, pre-treatment period from 19 to 21 January. The second collection was for a 5-day period (during and

immediately after treatment) from 21 to 26 January. Thereafter, collections were made every 14 days post-treatment until 21 March 2000. All workers, queens and varroa mites that were found on the white boards or in the dead bee traps from each sample were counted to calculate an average daily mortality rate for each collection period.

Room temperature and airflow

Each treatment room simulated the airflow conditions of a commercial wintering building with a fan jet air distribution system (Gruszka, 1998) but was modified to allow formic acid fumigation (Fig. 2.1). An air mixing chamber (Fig. 2.2) was designed to blow air through an air ventilation tube (Fig. 2.1d) at a rate of 106 L/s. Air was taken from the unheated attic of the building through a pipe (Fig. 2.2b) or recirculated from the treatment room itself (Fig. 2.2c). Baffles within pipes b and c (Fig. 2.2) allowed air flow direction to be controlled as necessary. Air was continuously distributed throughout the treatment room through an air distribution tube (6.7-cm radius; Fig. 2.1d) that ran the length of the treatment room and had 10 evenly spaced 5-cm-diameter holes. Air velocity from each hole was ≈ 5 m/s. Additional fans (Fig. 2.1a-c) sucked air out of the treatment room to provide continuous air exchange at a minimum rate of 0.24 L/s per hive (step 1 ventilation rate). As room temperature increased later in the winter, additional fans that were controlled by thermostats at 1 °C increments switched on in steps from 2 to 4 to bring the total maximum ventilation rate to 6.43 L/s per hive (Gruszka, 1998; Fig. 2.1a-c). The walls of each treatment room were covered with thin plastic (0.5-mil Polyester/3-mil Polyolefin Plastomer Coextrusion, Winpak Ltd., Winnipeg, Manitoba, Canada) to prevent air flow between the treatment rooms. In addition, the door of each treatment room was sealed for the 48 hours of treatment and

the subsequent 48 hours (22-26 January 2000). A baseboard heater (Fig. 2.1f) attached to a thermostat maintained each treatment room at a minimum temperature of 5 °C.

During treatment, each attic pipe baffle (Fig. 2.2b) was set to allow minimum airflow and each room pipe baffle (Fig. 2.2c) was set to allow maximum airflow. The vents for the step three ventilation fans (Fig. 2.1b) were sealed with thin plastic to prevent airflow between the treatment rooms. Only the minimum ventilation rate of 0.24 L/s per hive was allowed, thus, with the exception of heating, room temperature was not regulated during treatment.

Treatment

To distribute the formic acid throughout the treatment rooms, a fan and pan system was set up in the air mixing chamber (Fig. 2.2). A pan (25.0 by 25.0 by 5.5 cm; Fig. 2.2g) of liquid was placed in front of a fan (10-cm-diameter window fan, Holmes Products Corp., Milford, MA; Fig. 2.2f). A 10-liter plastic bag (Reliance Products Ltd., Winnipeg, Manitoba, Canada; Fig. 2.2a) was connected to the pan by a tube (0.4-cm inner diameter, Anderson Barrows Metals Corporation). 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 Limited, Brampton, Ontario, Canada).

Based upon previous testing in empty treatment rooms (unpublished data), various humidifier plates and dilutions of formic acid in the pan and reservoir bag were used to manipulate formic acid concentrations in the air of the treatment rooms. Three application methods were designed to expose bee colonies in different treatment rooms to different concentrations of formic acid, whereas the fourth treatment room was a control. The pan for the control room started with 1 L of water, a humidifier sponge (30 by 19 by

5 cm, Air-King Limited) and a bag with 8 L of water. The low-concentration treatment started with a pan containing 1 L of 65% formic acid, an identical humidifier sponge, and a bag with 8 L of 65% formic acid. The medium-concentration treatment started with a pan containing 1 L of 55% 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 bag with 8 L of 65% formic acid. The high-concentration treatment started with a pan containing 1 L of 85% formic acid, three identical humidifier plates and a bag with 8 L of 85% formic acid.

Formic Acid Sampling

Throughout the experiment, air samples were taken for formic acid concentration measurements using an air monitoring system (National Dräger, Pittsburgh, PA). Immediately before obtaining each sample, the entire volume of air was withdrawn from the sealed air-sampling tube and discarded using a disposable syringe. An air sample was then collected and the formic acid concentration measured by attaching a needle to a Dräger tube (Tube No. 6722101) with a piece of rubber tubing. The needle pierced the stopper and allowed air to be sucked into and through the Dräger tube and into the gas detector pump (Accuro 9022003). The volumes of air were drawn through the pump according to the manufacturer's instructions (National Dräger). The Dräger tube allowed an immediate measurement of acid concentration measured in ppm. Acid concentrations > 80 ppm were measured by using a smaller volume of air and extrapolated from a standard curve (Leichnetz, 1989).

Formic acid concentration was measured only in the three fumigated treatment rooms. Air samples were taken from the center of fumigated treatment rooms at heights

of 0.1, 0.5, and 1.5 m and from within hives 1-3 (Fig. 2.1) in each treatment room using the tubes at the center and edge of the honey bee cluster. Air samples were taken at 2, 6, and 12 hours at all locations. At 24, 48, and 72 h, measurements were taken at the edge and center of the cluster in hives 1-3, but only at the 0.5-m height in the center of each treatment room.

Temperature was measured every 10 min using HOBO[®] data loggers (Onset Computer Corporation) at the same sites as used for formic acid measurements. Additional temperature measurements were taken at the center and edge of the cluster in hives 4 and 5 (Fig. 2.1). Temperature was also monitored from similar sites in hives 1-5 (Fig. 2.1) in the control room.

Post-treatment

After 48 hours of treatment, the liquid formic acid solution was removed from the pans and the reservoir bags were emptied. The attic pipe baffles (Fig. 2.2b) were opened and the room pipe baffles (Fig. 2.2c) were closed to allow the maximum amount of fresh air into the treatment rooms. Additional ventilation fans were turned on to increase the air exchange to ≈ 0.71 L/s per hive (step 2 ventilation rate). The treatment rooms were ventilated in this way until 26 January 2000 when the door seals were removed and the fans were turned back to step 1. For the remainder of the winter, the fans were controlled by thermostats and activated in series when room temperatures rose above 5 °C according to standard practice (Gruszka, 1998).

After treatment ended, colonies were fed a 67 % sucrose solution *ad libitum* by using a hive-entrance feeder made of a 454-g plastic honey container. Small holes were pierced through the container, which was turned upside down. The sugar syrup solution

was captured in the lip of the lid where it was maintained by gravity and surface tension at a constant level.

On 23 March 2000, all hives were removed from the wintering building, weighed, and placed outside in the University of Manitoba apiary. Each hive was sampled the following day to assess worker cluster size, mean abundance of varroa mites and nosema spores, and tracheal mite prevalence as described above. When scoring cluster size in spring, an additional cluster size rank of zero was used to describe a dead colony.

Statistical analysis

A split-plot analysis of variance (ANOVA) design was used to analyze cluster size, mean abundance of varroa mites, and hive weight data that were collected at the beginning and end of the experiment and was also used to analyze data for daily worker bee and varroa mite mortality throughout the winter (PROC GLM; SAS Institute Inc., 1999). These tests used treatment room and block as main plot factors and time as the subplot factor. Treatment room x block was used as the error term for the main effects. Data on the mean abundance of varroa mites and tracheal mite prevalence were analyzed after arcsine transformation. For varroa mean abundance, division by 103 was necessary because mite levels of up to 103 mites per 100 bees were recorded. Mean abundance of nosema spores was analyzed after log transformation [$\log_{10}(x + 0.05)$]. When significant treatment differences were found ($P < 0.05$), a Student-Newman-Keuls' multiple range test was used to compare differences between means. When significant treatment room x time interactions occurred, separate ANOVA's were conducted at each time interval. For nosema spores and varroa mites sampled before and after the winter, contrasts were used to compare the change in mean abundance in each treatment room to the change in the

control using Bonferroni-corrected alpha values. Colony and queen survival data were analyzed using Fisher's exact test (PROC FREQ; SAS Institute Inc., 1999).

Comparisons of formic acid concentration levels and temperatures based on height and position of measurement were analyzed using a split-split-plot design. Treatment room was the main plot factor, height, and position (treatment room versus edge versus center of the honey bee cluster) were the subplot factors, and time was the sub-subplot factor. Treatment room x position was used as the error term for treatment room and position effects. Treatment room x position x height was used as the error term for height, treatment room x location, treatment room x height, and location x height. When significant differences ($P < 0.05$) were found, a Student-Newman-Keuls multiple range test was used to compare differences between means. Correlations between colony survival, colony weight, and cluster size were analyzed using PROC CORR (SAS Institute Inc., 1999).

RESULTS

Formic acid concentration

The mean concentration of formic acid in the room during treatment in the low-concentration treatment room was 11.9 ± 1.2 ppm. The medium-concentration treatment room had a mean of 25.8 ± 1.4 ppm, and the high-concentration treatment room had a mean of 41.2 ± 3.3 ppm during treatment. Formic acid was well distributed throughout each treatment room. There was no significant difference between measurements taken at different heights ($F = 0.04$, $df = 2, 8$, $P > 0.05$; Fig. 2.3) and no interaction between

height and location as measured at sites within the hives or in the treatment room air ($F = 0.76$, $df = 4, 8$, $P > 0.05$; Fig. 2.3).

Formic acid concentration in the hives was significantly lower than the acid concentrations in the treatment rooms during treatment ($F = 25.20$, $df = 2, 5$, $P < 0.001$; Student-Newman-Keuls; Fig. 2.4). Within the hives, the acid concentration did not differ significantly between the edge and the center of the honey bee cluster (Student-Newman-Keuls). After the acid was removed from the air mixing chamber, the concentration of acid in the hives remained relatively stable for at least 24 h, whereas the acid levels in the treatment rooms dropped rapidly (Fig. 2.4).

***V. destructor* mortality**

Average daily mite fall was affected by treatment room as indicated by a significant treatment room x time interaction ($F = 9.02$, $df = 15, 410$, $P < 0.0001$; Fig. 2.5A). During the treatment period, average daily mite fall was significantly affected by treatment room ($F = 9.89$, $df = 3, 58$, $P < 0.001$). The high-concentration treatment room had significantly more mortality than the control and the medium- and low-concentration treatment rooms were not significantly different from any other treatment room (Student-Newman-Keuls). During pre- and post-treatment periods, there was no significant difference in average daily mite fall between treatment rooms (Student-Newman-Keuls).

Worker bee mortality

Honey bee worker mortality was not affected by treatment as indicated by a non-significant treatment room x time interaction ($F = 1.36$, $df = 15, 410$, $P > 0.05$; Fig. 2.5B). There was no effect of treatment room on honey bee mortality during the pre-treatment, treatment, or post-treatment periods.

Queen bee mortality

There was a significant difference in the rate of queen loss between treatment rooms ($P < 0.01$). The high concentration killed significantly more queens than the control ($P < 0.02$). In the high-concentration treatment room, five of 21 (24%) queens fell during the treatment sampling period and an additional two (9.5%) were lost after treatment. The control and medium-concentration treatment rooms also lost one queen (4.8%) each during the experiment, but no queens were lost in the low-concentration treatment room.

Mean V. destructor abundance

Mean *V. destructor* abundance was affected by treatment room as indicated by a significant treatment room x time interaction ($F = 10.37$, $df = 3, 39$, $P < 0.001$). The drop in the mean abundance of varroa mites over the winter in colonies in the high-concentration treatment room was significantly greater than in the control colonies ($F = 7.36$, $df = 1, 39$, $P < 0.01$; Fig. 2.6A). However, there were no significant differences between the change in mean abundance over the winter in the low- and medium-concentration treatment rooms relative to the control (low; $F = 2.28$, $df = 1, 39$, $P > 0.05$; medium; $F = 0.00$, $df = 1, 39$, $P > 0.05$). Before treatment, there was no significant difference in mean abundance of varroa mites between treatment rooms ($F = 1.03$, $df = 3, 60$, $P > 0.05$).

Mean N. apis spore abundance

Nosema spore abundance was affected by treatment room as indicated by a significant treatment room x time interaction ($F = 2.27$, $df = 3, 39$, $P < 0.05$; Fig. 2.6B). The change in the mean abundance of nosema spores over time in the high-concentration

treatment room colonies was significantly different than in the control colonies ($F = 5.07$, $df = 1, 39$, $P < 0.05$). In the control, low- and medium-concentration treatment rooms, the mean abundance of nosema spores increased over the course of the winter, whereas in the high-concentration treatment room, levels decreased slightly (Fig. 2.6B).

A. woodi prevalence

Tracheal mite prevalence was not affected by treatment room as indicated by a nonsignificant treatment room x time interaction ($F = 0.64$, $df = 3, 104$, $P > 0.05$; Fig. 2.6C).

Colony weight

Colony weight was not affected by treatment room as indicated by a nonsignificant treatment room x time interaction ($F = 0.06$, $df = 3, 125$, $P > 0.05$). However, hive weight decreased significantly in colonies in all treatment rooms over the winter ($F = 327.79$, $df = 1, 125$, $P < 0.001$; Table 2.1). The differences in colony weight were not correlated with colony survival ($P > 0.05$). The amount of sugar syrup taken in from hive entrance feeders after fumigation ranged from 0 to 1.5 kg per hive and was not affected by treatment room ($F = 0.47$, $df = 3, 60$, $P > 0.05$).

Cluster size

Cluster size was not affected by treatment room as indicated by a nonsignificant treatment room x time interaction ($F = 1.72$, $df = 3, 125$, $P > 0.05$). However, the average cluster size decreased significantly over the winter ($F = 6.08$, $df = 1, 125$, $P < 0.02$; Table 2.1).

Temperature

During the 48 hours of fumigation, the mean temperature of the honey bee cluster was 18.83 ± 0.14 °C in the control, 22.53 ± 0.14 °C in the low-concentration, 20.59 ± 0.22 °C in the medium-concentration, and 22.95 ± 0.25 °C in the high-concentration treatment; an overall mean of 21.17 ± 0.10 °C. The mean temperature at the edge of the cluster was 7.20 ± 0.09 °C in the control, 7.22 ± 0.06 °C in the low-concentration, 7.66 ± 0.06 °C in the medium-concentration, and 7.87 ± 0.04 °C in the high-concentration treatment; an overall mean of 7.46 ± 0.04 °C. Treatment room did not effect the temperature in the center ($F = 0.22$, $df = 3, 10$, $P > 0.05$) or the edge of the cluster ($F = 0.33$, $df = 3, 10$, $P > 0.05$).

Colony survival

Treatment room did not significantly affect colony survival ($P > 0.05$). In the spring, the control and high-concentration treatment rooms contained 14 surviving colonies, whereas the low-concentration treatment contained 17 and the medium-concentration room contained 18.

DISCUSSION

Indoor winter fumigation of varroa-infested honey bee colonies with formic acid is feasible. Indoor fumigation did not affect worker bee mortality, but varroa mite mortality at all three acid concentrations tended to be higher than in the control, although the tendency was only significant for the high concentration. In the high-concentration treatment room, the mean abundance of varroa mites was reduced and the development of nosema spores was suppressed. Formic acid treatment did not negatively effect overall

colony survival and did not have a detectable effect on tracheal mite prevalence.

Colonies exposed to high concentrations of formic acid experienced significant queen loss. These results suggest that large-scale fumigation with formic acid can be effective, but more research is required before this treatment can be used commercially.

Formic acid has a higher vapor density than air (1.59 and 1.0, respectively), and thus might be expected to accumulate in the lower part of the treatment room (CCOHS, 2002). However, our results show that when using an air tube ventilation system, formic acid vapors were evenly distributed at heights of up to 1.5 m. The ventilation system used in this experiment was designed to simulate those recommended for a commercial wintering building (Gruszka, 1998). Therefore, it might be possible for beekeepers to keep their hives stacked during fumigation without significant modifications to building ventilation. In commercial buildings, colonies are often stacked much higher than three high, so further testing of formic acid distribution in the air in individual facilities with different ventilation systems will be required.

The formic acid concentration in the air within each of our treatment rooms was significantly higher than that in the hives contained within the rooms. In addition, the acid concentration in hives remained stable for at least 24 hours after the acid was removed from the air mixing chambers (Fig. 2.4). Elucidating the cause of the difference between the room and hive air may be important for standardization of this method. Honey absorbs acid (Stoya et al., 1986; Liu, 1992; Liu et al., 1993) and sorption of formic acid by honey, hive parts, or bees themselves could cause the low concentrations of acid measured in the hive air. Release of acid from these acid sinks after the treatment

has been terminated may continue the fumigation for longer than expected and will have to be considered when calculating the concentration received by each colony.

During treatment, mite drop increased with concentration of formic acid, as expected (Underwood and Currie, 2003). However, only hives in the high-concentration treatment room had a mean abundance of varroa mites in spring that was less than 1 mite per 100 bees, the point at which economic losses occur in the prairie region of Canada (Gatien and Currie, 2003). Although there was increased varroa mite mortality in the low- and medium-concentration treatment rooms, this was not statistically significant and the mean abundance of varroa mites at the end of the winter did not differ from that found in the control colonies. The short duration of fumigation is suspected to be the cause of the low efficacy in low- and medium-concentration treatment rooms. Queen loss in these treatment rooms was also very low, suggesting that these concentrations of acid may be acceptable if longer exposure times were used.

Collections from white board samples and dead bee traps showed that fumigation with formic acid in winter did not significantly increase worker bee mortality, regardless of concentration. Colony survival, cluster size, hive weight, and syrup intake were also not affected by treatment room.

Although our experimental design did not allow us to separate formic acid treatment effects from possible room effects, we assume that room effects were negligible because the rooms were monitored and controlled for temperature, ventilation, and light. We also took pre-treatment samples from all colonies in each room and these indicated that there were no pre-treatment differences between treatment rooms. In

addition, treatment differences for all variables were reported only when significant treatment room x time interactions were present (Oksanen, 2001).

More than 30 % of queens were lost in the high-concentration treatment room, and this would be unacceptable for commercial beekeepers. Queen loss associated with formic acid use has been seen in previous studies (VonPoseern, 1988; Fries, 1989), but these losses are usually associated with high concentrations of formic acid in combination with high ambient temperatures ($> 25^{\circ}\text{C}$). The factors resulting in queen loss in our study have not been determined, but were not related to high ambient temperatures, because cluster temperatures averaged only $18.83 - 22.95^{\circ}\text{C}$. Future studies are needed to elucidate the cause of queen loss and to develop strategies to prevent it.

This study showed significant suppression of nosema spore buildup with formic acid over winter, but only in the high-concentration treatment room. Although the mode of action of formic acid on nosema is unknown, we suspect that formic acid fumigation either alters the honey bee gut, indirectly affecting the progression of nosema disease, or renders spores inviable, thus reducing infection rates. The latter seems more likely, because attempts to control nosema by altering gut pH have not been successful (Gontarski, 1951). Acetic acid is effective at killing nosema spores (deRuijter and van der Steen, 1989), and it is possible that formic acid may work in a similar manner if the correct concentration is achieved. In outdoor spring applications, neither pour-on nor slow release treatments of nosema-infected honey bees with formic acid show an effect on mean nosema spore abundance (Ostermann, 2002). In addition, fumigation of feces-stained comb with 85% formic acid does not suppress nosema disease (Fries, 1990). The combination of exposure time and concentration may be important in affecting the ability

of formic acid to control nosema, and it is likely that this combination is not achieved in field applications or that temperature differences may affect treatment efficacy.

Formic acid is usually effective in killing tracheal mites. For example, Hoppe et al. (1989) found good control of tracheal mites using a fall treatment and Wilson et al. (1993) reported good control of tracheal mites by using a variety of formic acid concentrations and treatment intervals. However, in this study, formic acid treatment did not affect tracheal mite prevalence. The short duration of fumigation may have reduced its efficacy or perhaps our methods were not sensitive enough to detect changes in mite mortality. The effect of formic acid on tracheal mites was assessed counting the number of bees with infested tracheae. Thus, we were not able to discern live from dead mites. Examination of live bees would have been necessary to distinguish between bees infested with live mites and those with mites killed by formic acid treatment, but remaining in the tracheae. Future research should involve examination of live bees to determine the effectiveness of winter fumigation in controlling tracheal mites.

Introduction of an alternative varroa mite control technique that is economical and effective is important for commercial honey producers and is especially critical in light of growing concerns about mite resistance to acaricides. Formic acid fumigation of indoor-wintered colonies will save labor costs and may reduce chemical costs, depending on the concentration that is required. Although applicator safety will be a concern, there are some advantages over existing methods. Because all hives can be treated simultaneously, the amount of chemical handling would be greatly decreased as compared to what is required to treat thousands of individual colonies outdoors. Thus, the probability that the applicator will be injured through accidental spills would decrease. Producers that winter

indoors could readily incorporate this technique, as little modification of buildings would be required.

The results of this study show that indoor winter fumigation with formic acid is feasible. Formic acid was easily distributed throughout the treatment rooms and concentrations high enough to reduce varroa mite populations to an acceptable level were attained. Colonies exposed to average concentrations of 42 ppm for 2 days had reduced levels of varroa mites and suppressed development of nosema disease relative to untreated colonies. However, this high-concentration treatment caused excessive queen loss. Future studies will be required to determine the exact concentration, timing, and/or number of treatments required to reliably bring the mean abundance of varroa mites below economic threshold levels. Further studies will also have to clarify whether the same formic acid treatments will also be effective against tracheal mites and nosema disease. This will help to optimize this mite control method and make it suitable for use in commercial operations. Developing a treatment method that reduces or eliminates queen loss will be critical for the adoption of this treatment practice.

Table 2.1. Mean cluster ranking and hive weight (\pm SE) measured before and after wintering in colonies that were untreated (control) or treated with a low, medium, or high concentration of formic acid for 48 hours (before; $n = 21$ colonies in each treatment group; after; control and high $n = 14$, low $n = 17$, medium $n = 18$ colonies). No significant treatment room x time interaction was found for cluster size or hive weight.

<i>Treatment room</i>	<i>Cluster size (rank)</i>		<i>Hive weight (kg)</i>	
	Before (\pm SE)	After (\pm SE)	Before (\pm SE)	After (\pm SE)
Control	2.00 \pm 0.14	1.29 \pm 0.25	32.4 \pm 0.97	25.6 \pm 0.93
Low	1.95 \pm 0.15	1.62 \pm 0.25	31.3 \pm 0.97	24.1 \pm 0.97
Medium	1.71 \pm 0.12	1.81 \pm 0.22	32.4 \pm 0.89	25.4 \pm 0.99
High	1.86 \pm 0.16	1.48 \pm 0.26	31.4 \pm 0.81	24.0 \pm 0.97

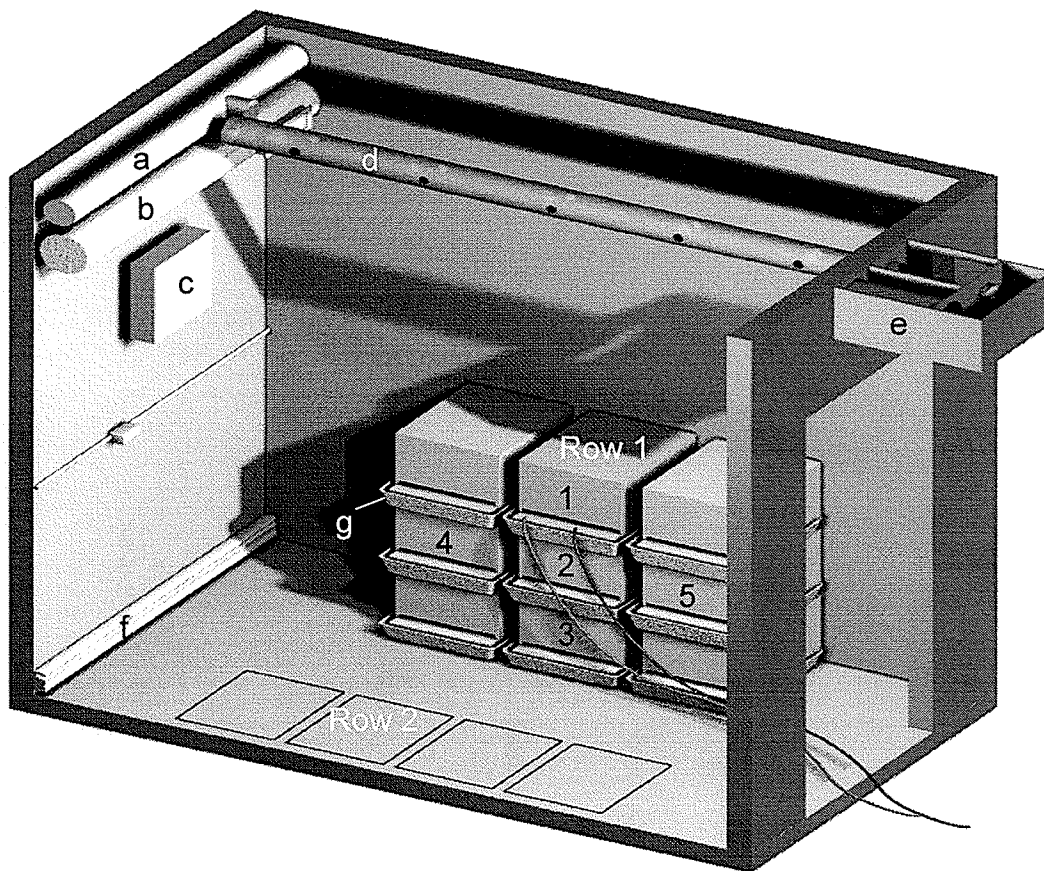


Figure 2.1. Setup of a treatment room. Missing from the diagram: 12 hives in row 2, tubes coming from hives 2 and 3, and tubes used to measure the formic acid concentration in the center of the room. (a) Ventilation tube for step 1 and 2 exhaust fans. (b) Ventilation tube for step 3 exhaust fan. (c) Ventilation light shield for step 4 exhaust fan (Gruszka, 1998). (d) Plastic air distribution duct for air mixing and formic acid distribution. (e) Air mixing chamber (see Fig. 2.2). (f) Baseboard heater. (g) Dead bee trap. Hives 1 through 5 were monitored with air and/or temperature monitoring devices (see Materials and Methods).

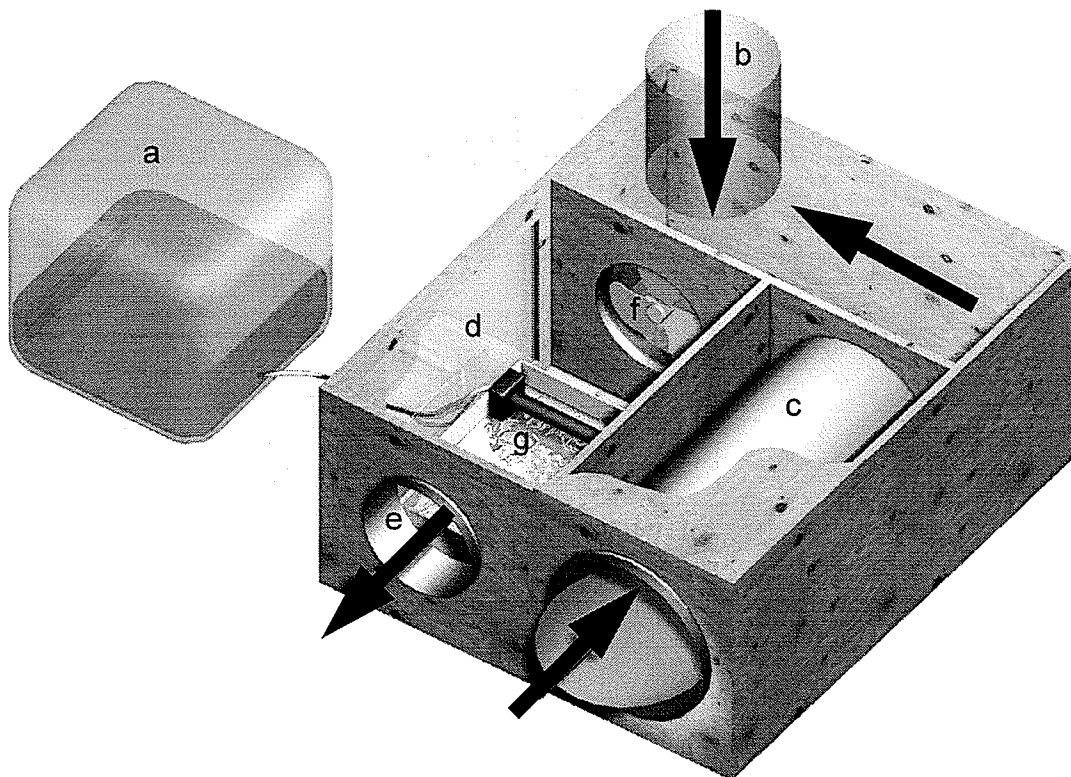


Figure 2.2. Air mixing chamber. (a) Reservoir bag. (b) Attic pipe to allow fresh air into the room. (c) Room pipe to allow room air to be recirculated. (d) Window for addition and removal of formic acid. (e) Connects mixing chamber with air distribution tube in the treatment room (see Fig. 1d). (f) Fan. (g) Pan containing liquid and a float valve. Arrows indicate the direction of airflow.

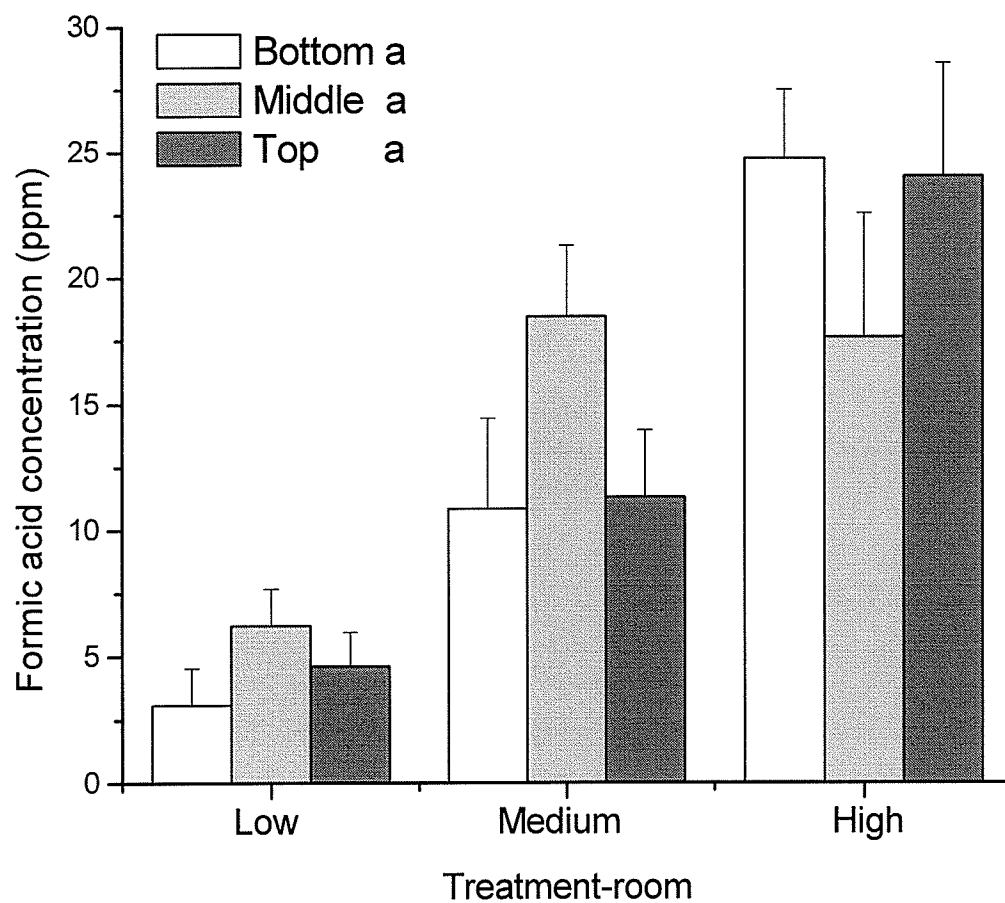


Figure 2.3. Mean formic acid concentration (\pm SE) in the bottom, middle, and top sample sites of hives stacked three high. Each bar represents the mean of four samples taken during the 48 hours of fumigation. Sampling sites followed by the same letter are not significantly different (Student-Newman-Keuls; $P > 0.05$).

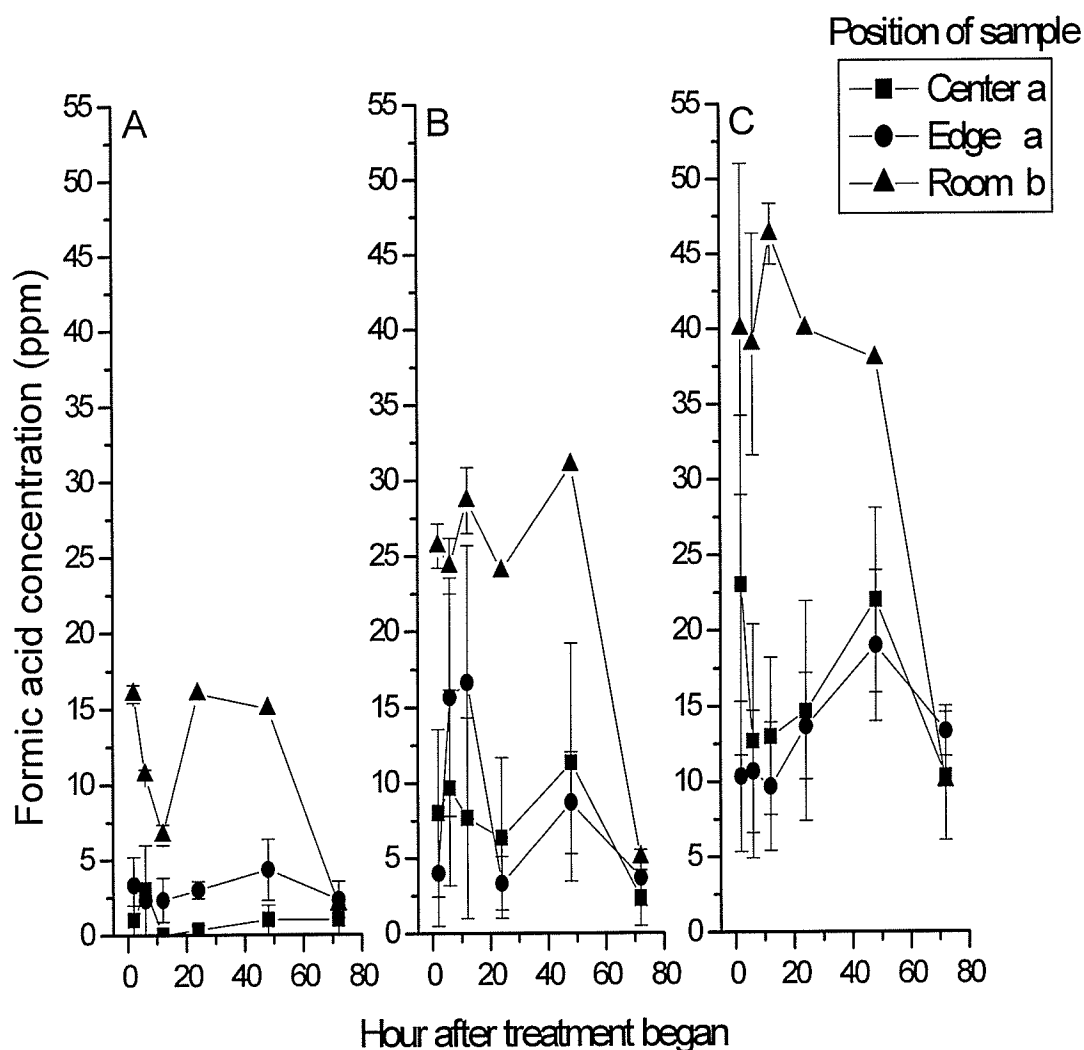


Figure 2.4. Mean formic acid concentration in air (\pm SE) of treatment rooms (room) and from the center (center), and edge of the cluster (edge) within hives during (0-48 h) and after (72 h) fumigation. Each point represents an average of the three measurement sites. Room measurements after hour 12 represent only one measurement. (A) Low-concentration treatment room. (B) Medium-concentration treatment room. (C) High-concentration treatment room. Means followed by the same letter are not significantly different within treatment rooms during fumigation (Student-Newman-Keuls; $P > 0.05$).

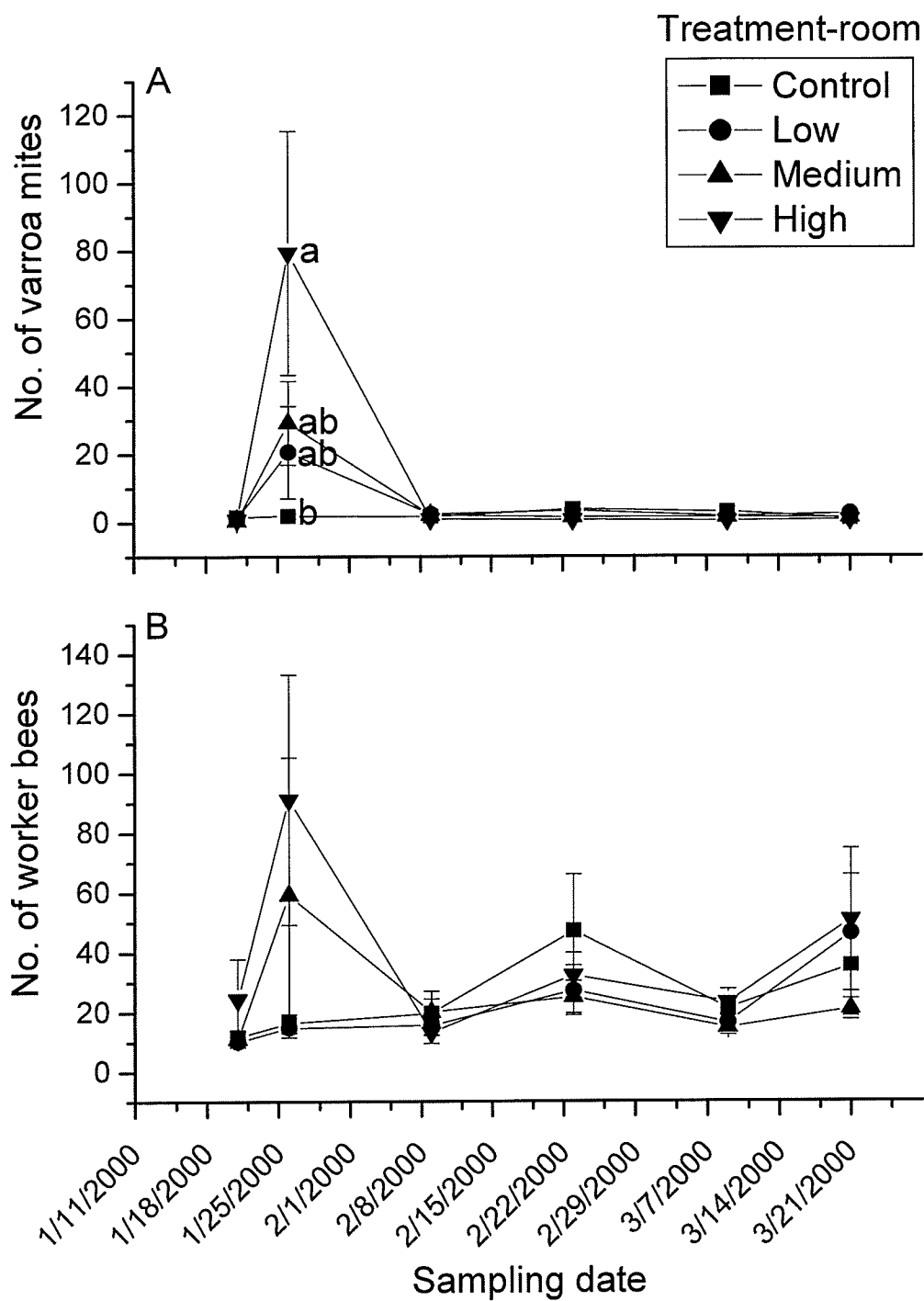


Figure 2.5. Mean number of varroa mites (A) or worker honey bees (B) falling each day (\pm SE) from colonies that were untreated (control) or treated with a low, medium, or high concentration of formic acid for 48 hours ($n = 21$ colonies per treatment group). Mites and bees were collected from white boards placed on the bottom

board of each hive and dead bee traps. Means from the treatment period (21-26 January) followed by the same letter are not significantly different from each other (Student-Newman-Keuls; $P > 0.05$). Means from the pre-treatment (19-21 January) and post-treatment periods (26 January–21 March) are not significantly different from each other (Student-Newman-Keuls; $P > 0.05$).

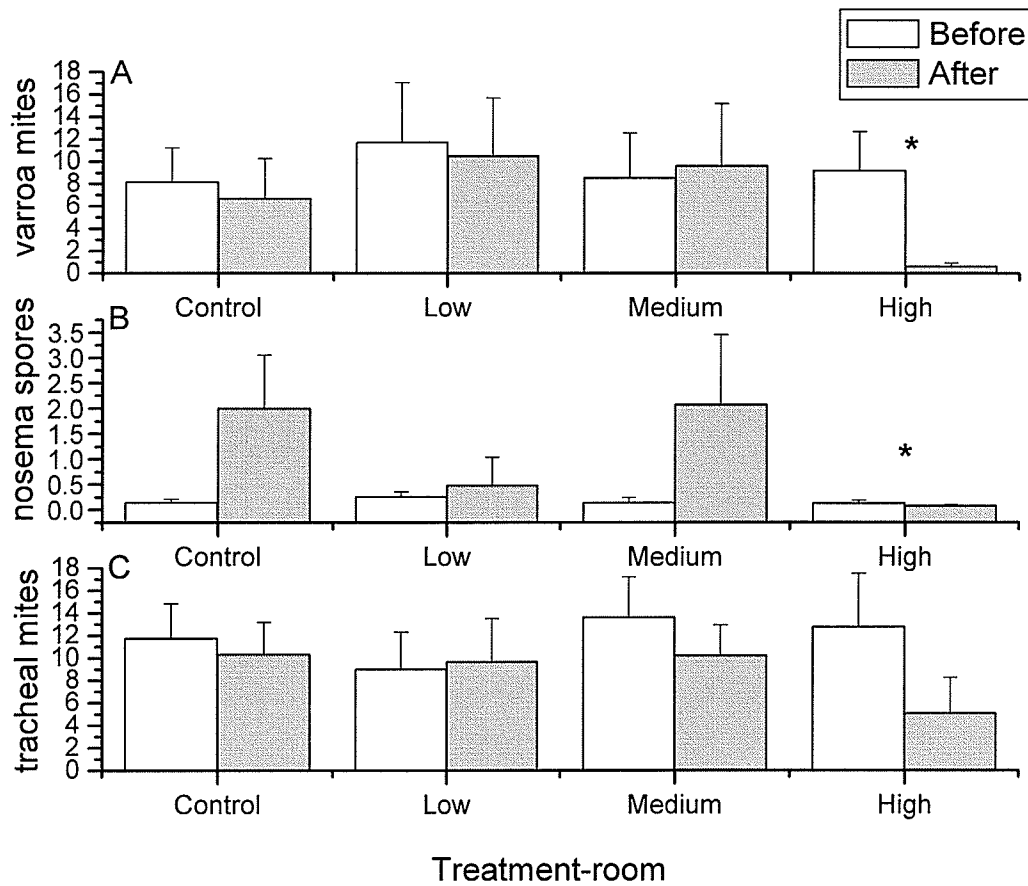


Figure 2.6. Varroa mite mean abundance (no. of mites per 100 bees \pm SE) (A), nosema spore mean abundance (millions of spores per bee \pm SE) (B), or tracheal mite prevalence (no. of infested bees per 100 bees \pm SE) (C) measured before (before) and after (after) wintering in colonies that were untreated (control) or treated with a low, medium, or high concentration of formic acid for 48 hours (before; $n = 21$ colonies in each treatment group; after; control and high $n = 14$, low $n = 17$, medium $n = 18$ colonies). An asterisk indicates a significant treatment x time interaction for each concentration compared with the control ($P < 0.05$).

CHAPTER 3. INDOOR WINTER FUMIGATION OF HONEY BEES WITH FORMIC ACID: A LOW TEMPERATURE CUMULATIVE CONCENTRATION-RESPONSE FOR VARROA MITES AND A POSSIBLE QUEEN LOSS PREVENTION STRATEGY

ABSTRACT

This study established a cumulative concentration-mortality relationship for high concentration formic acid fumigation of varroa mites in colonies held at 2 - 4 °C, examined the effect of the formic acid release pattern on queen loss and compared the effect of ventilation rate on queen loss and treatment efficacy during fumigation in two experiments conducted in a wintering building. In experiment 1, 50 % and 95 % of varroa mites were killed when exposed to concentration x time combinations of 49.3 ppm*days (CT₅₀ product) and 111.2 ppm*days (CT₉₅ product) of formic acid in hive air, respectively. No queen loss was observed. In experiment 2, 33 % of queens were lost when minimum ventilation was used with high-concentration fumigation whereas no queens were lost in controls or colonies exposed to high-concentration fumigation with variable ventilation. Queen loss was associated with in-hive peak formic acid concentration measurement, but not CT product, suggesting that queens are affected by acute rather than chronic toxicity to formic acid.

INTRODUCTION

Indoor wintering of honey bees, *Apis mellifera* L., is common in the northern prairie region of North America (Gruszka, 1998). Formic acid fumigation of honey bee colonies in indoor wintering facilities is a feasible alternative varroa mite, *Varroa destructor* Anderson and Trueman, control method in northern climates (Underwood and Currie, 2004). This control method is highly desirable because it exploits the pause in bee and mite reproduction that occurs in winter (Harris, 1980), allows ambient conditions to be controlled (Gruszka, 1998), and will likely reduce labor costs compared to conventional application methods. Before indoor winter fumigation can be applied on a commercial scale, questions related to the distribution of formic acid throughout the room, variation in concentrations within the hives, the most effective concentration, and queen loss need to be addressed.

During indoor fumigation, formic acid is evenly distributed both within the room and in hives at heights up to 1.5 m (the equivalent of the height of three single-chambered colonies) (Underwood and Currie, 2004). Because commercial beekeepers commonly stack their hives much higher while wintering them indoors, information about the distribution of formic acid to hives stacked more than three high is necessary. During winter fumigation, the concentration of acid in the hive air is significantly lower than that measured in the treatment room air, but it is not known whether this may be caused by sorption of acid into honey or bees, or whether room air circulation may influence movement of air into hives (Underwood and Currie, 2004). Understanding the relationship between room and hive air and how this affects the calculation of treatment

efficacy will aid in the standardization of the concentration that each colony receives and will increase the precision of this treatment.

Queen loss during indoor fumigation with formic acid is also a problem. It has been speculated that high ambient temperatures and high concentrations of formic acid are associated with queen loss during outdoor applications of formic acid (VonPosern, 1988). However, substantial queen loss can occur at moderate concentrations of formic acid when ambient temperatures are low during indoor fumigation in winter (Underwood and Currie, 2004). Underwood and Currie (2004) showed that indoor fumigation for 48 hours with 40 ppm formic acid in the room air can cause 33% queen mortality. The reason for this queen loss was not determined but it might be an acute toxic effect associated with exposure to a sudden high concentration of formic acid. If this is true and the concentration of acid can be increased slowly to a high concentration, or be kept below a lethal limit, the incidence of queen loss may be reduced.

The main objectives of this study were to 1) establish a cumulative concentration-mortality relationship for formic acid fumigation of varroa mites in colonies held at 2 - 4 °C, 2) to examine the effect of the pattern of formic acid release on queen loss and 3) to compare the effects of two ventilation rates on queen loss and treatment efficacy during formic acid fumigation. These objectives were addressed through two experiments. The specific objectives of experiment 1 were 1) to establish a concentration-response curve for in-hive formic acid concentration and varroa mite mortality over time and 2) to determine the relative effect of a high-concentration spike fumigation and a constant high-concentration fumigation on queen survival. The specific objectives of experiment 2 were 1) to test whether room ventilation settings affect queen survival in full-size

colonies fumigated with a high concentration of formic acid and 2) to examine the factors that affect variability in formic acid concentration among hives and how they might influence queen loss and treatment efficacy.

MATERIALS AND METHODS

Experimental treatment rooms and general fumigation methods

Fumigation tests were conducted in four 3.0 x 1.7 x 2.5 m treatment rooms in the wintering building at the University of Manitoba in Winnipeg, Manitoba, Canada (N 49° 48' 32", W 97° 07' 37"). Each room simulated the air flow conditions of a standard commercial wintering building with a fan jet distribution system (Gruszka, 1998), but was modified to allow formic acid fumigation (Underwood and Currie, 2004). 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 x 25.0 x 5.5 cm) of liquid containing three T-shaped humidifier plates (11.5 x 16.7 x 0.2 cm, 64-3119-0, Dundas-Jafine Industries Ltd., Weston, ON, Canada) was placed in front of a fan (10 cm diameter window fan, Holmes Products Corp., Milford, MA, USA). A 10-liter plastic bag (Reliance Products Ltd., Winnipeg, MB, Canada) was connected to the pan by a polyethylene tube (4.3 mm inner diameter, Anderson Barrows Metals Corp., Palmdale, CA, USA). 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, ON, Canada). In both experiments, all rooms received a constant minimum ventilation rate of 0.4 L/s/m³.

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, USA) calibrated using Dräger tubes (Tube No. 6722101 with Accuro 9022003 gas detector pump, National Dräger, Pittsburgh, PA, USA) or by using Dräger tubes directly. 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 Scientific Ltd., Ottawa, ON, Canada). Measurements were taken from within hives at a point near the center of the honey bee cluster and from the room at a height of 1.5 m from 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. A HOBO[®] data logger (Onset Computer Corp., Bourne, MA, USA) was placed in the center of each treatment room near the opening of the air-sampling tube, where temperature and humidity were measured every 30 min throughout the treatment period for experiments 1 and 2.

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

Experiment 1

In late July 2001, 30 queenless 5-frame nuclear colonies were established in an apiary on the University of Manitoba campus. In early August, a queen cell was added to each colony. Queens were open-mated and laying eggs by late August. On 30 November, the hives were brought indoors and randomly assigned to one of two experimental treatment rooms. Each hive had a completely open bottom entrance (37 x 2.5 cm) and a telescoping lid. A white board covering the entire surface of the bottom board was placed in each hive to collect all queen bees and varroa mites falling from the cluster. The hives were stacked three high against one wall in five adjacent columns. Ventilation in both rooms was held at the minimum ventilation rate throughout the experiment (as described above).

On 17 December, formic acid fumigation began. One treatment room received a constant high concentration of formic acid and the second room received a low concentration with a high-concentration spike on the third day of fumigation. In the constant high-concentration treatment room, fumigation began with 1 L 85% formic acid in the pan and 5 L 85% formic acid in the reservoir bag that maintained the flow of 85% formic acid for the duration of the treatment. In the high-concentration spike treatment room, fumigation began with 1 L water in the pan and 5 L 65% formic acid in the reservoir bag. After 72 h, the bag contents were replaced with 85% formic acid. After an additional 12 h, an extra pan containing 1.5 L 90% formic acid was placed in the air mixing chamber (Underwood and Currie, 2004). Fumigation in both treatment rooms was stopped after 5 days on 22 December by removing the formic acid from the pan and increasing the fresh air intake.

For the first 3 days of fumigation, white board contents were collected every 6 h. For the following 4 days collections were at 12-hour intervals and for the remaining 5 days collections were at 24-hour intervals. The concentration of formic acid in the room air and in the air from six hives per treatment room was also measured at these intervals. Formic acid concentration was monitored in the hives in the center column and in the three hives in the column farthest from the door. The remaining hives in each treatment room were not fitted with air-sampling tubes and were not monitored. After fumigation, one fluvalinate strip was added to each hive. White board counts for a 48-hour fluvalinate treatment were used to determine the number of varroa mites remaining after treatment. This number was then used to obtain proportional mite mortality values by dividing the cumulative daily mite mortality to day i by the total number of mites that dropped during the entire trial. These colonies had never been exposed to fluvalinate, so the mites were considered to be susceptible to it.

Experiment 2

Eighty-four single-chamber Langstroth hives were obtained from nine commercial beekeepers during the fall of 2001. Pre-treatment samples were collected on 5 November to estimate the mean abundance of varroa mites and the size of the honey bee cluster. Mean abundance of varroa mites was measured from a sample of 200-300 workers from each colony by the alcohol wash method (Gatien and Currie, 2003). Cluster size was estimated as the number of frames completely covered with bees.

On 11 December 2001, all hives were fitted with air-sampling tubes (as described above). Colonies were weighed and brought indoors where they were positioned in stacks placed randomly in a large holding room (5.2 x 7.2 x 2.5 m). Each hive had a

completely open bottom entrance (37 x 2.5 cm), but lid type varied with beekeeper. On 27 February 2002, the colonies were randomly assigned to four experimental treatment rooms. A white board covering the entire surface of the bottom board was placed in each hive to collect any queen bees falling from the cluster. In each treatment room, the hives were arranged 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.

Experimental treatments consisted of two fumigation levels; fumigated and unfumigated. Within each level of fumigation, pairs were created based on ventilation level. One pair had only the minimum ventilation (as described above) while the other pair had variable ventilation with the minimum ventilation supplemented with an additional fan set on a thermostat that increased the ventilation rate to 8.2 L/s/m³ when room temperature rose above 6 °C. Acid fumigation was applied using the method for the constant high-concentration treatment in experiment 1. Fumigation began on 4 March and the acid was removed from the air mixing chamber on 11 March after 7 days of fumigation.

During fumigation, all white board samples were collected daily. White board contents were assessed only to determine the presence or absence of fallen queens. After fumigation ceased, samples were collected every two weeks. The formic acid concentration in the room air and in the air of each hive in fumigated rooms was also measured (as described above). Because of the large number of hives tested and the length of time required to collect gas samples using the *PortaSens II* sampling device, all hives could not be sampled in a single day. Therefore, seven hives per fumigated treatment room were sampled each day so that each hive was sampled on a 3-day

rotation. In addition, all hives were sampled using Dräger tubes on the seventh day. Room air in both fumigated rooms was sampled daily using the *PortaSens II* sampling device. Air samples were not obtained on day 4 in the variable-ventilation treatment room and on day 2 in the minimum-ventilation treatment room.

Colonies were moved outdoors on 8 April at which time post-treatment measurements of hive weight, the size of the honey bee cluster and the mean abundance of varroa mites were obtained as described above. The total number of mites remaining in the colonies at the end of the winter was estimated by multiplying the mean abundance of varroa mites from the alcohol wash by the size of the honey bee population. This number was then used to obtain proportional mite mortality values by dividing the cumulative daily mite mortality to day *i* by the total number of mites that dropped during the entire trial. Little or no brood was present in colonies on the day they were moved out of the wintering building. In addition, queen status was assessed in all surviving colonies by observation of the presence of eggs, brood, or the queen herself on 17 April.

Statistical Analysis

The relationship between in-hive CT product (measured daily) and the associated cumulative varroa mite mortality in experiment 1 was determined using the pooled data from all 12 monitored hives in both treatment rooms. This created a line that represents the proportion of all mites surviving until each CT product was achieved. The CT₅₀ and CT₉₅ products were extracted from the line and are the CT product that was achieved when 50 and 95 % of all mites died, respectively. Differences in the overall response in the two treatment rooms were examined using the Log-rank and Wilcoxon tests of the

LIFETEST procedure using pooled data from the six hives that were monitored within each treatment room (SAS Institute, Inc., 1999).

Experiment 2 was set up using a Before-After Control-Impact (BACI) design (Stewart-Oaten et al., 1986; Smith, 2002) in which hives were treated as replicates. A split-plot factorial analysis of variance was where fumigation level and ventilation level were main plot factors and time was the sub-plot factor. Changes in varroa mite mean abundance, cluster size and hive weight were assessed using fumigation treatment x time or ventilation treatment x time interactions (Snedecor and Cochran, 1980). Because varroa mite mean abundance values are proportions, these data were analyzed after arcsine transformation.

The effect of treatment room on queen bee survival in experiment 2 was analyzed using the Log-rank and Wilcoxon tests of the LIFETEST procedure (SAS Institute Inc., 1999). The effect of peak formic acid concentration and cumulative concentration within each hive on queen mortality was analyzed using Fisher's exact test (PROC FREQ; SAS Institute Inc., 1999). Hives were grouped into categories based on the level of formic acid in the hive. For CT product data, the four categories were in 50 ppm*days increments with the final category being all values ≥ 150 ppm*days. For peak formic acid concentration measurements, the three categories were in 10 ppm increments with the final category being all values ≥ 20 ppm.

The effects of cluster size and hive weight on in-hive CT product in experiment 2 were analyzed using PROC CORR (SAS Institute Inc., 1999). Differences in CT products based on hive placement within each formic acid treated room were analyzed

using a randomized complete block analysis of variance with row (hive height) as the blocking factor (PROC GLM; SAS Institute Inc., 1999).

RESULTS

Experiment 1

During fumigation, formic acid concentration in the hive air was lower than in the room air and there was considerable variation in the cumulative concentration (CT product) that each hive received (Fig. 3.1). In the high-concentration spike treatment room, formic acid concentration in the room air averaged 4.6 ± 0.7 ppm (range 3.2 - 10.6 ppm) during the first 3 days and increased to an average of 47.6 ± 7.4 ppm (range 40.2 - 55.0 ppm) during the final 2 days of fumigation. In the constant high-concentration treatment room, formic acid concentration averaged 62.5 ± 2.4 ppm (range 48.1 - 75.4 ppm) throughout the 5-day fumigation period. In-hive measurements of formic acid concentration averaged only 3.2 ± 0.2 ppm (range 2.5 - 13.7 ppm) and 5.0 ± 0.3 ppm (range 2.0 - 18.0 ppm) in the high-concentration spike treatment and constant high-concentration treatment, respectively.

Daily mite drop over the course of fumigation differed between the high-concentration spike and constant high-concentration treatments in both early and late time periods (Log-Rank, $P < 0.0001$; Wilcoxon, $P < 0.0001$; Fig. 3.2). Final mite mortality averaged over all hives was 51.5 ± 10.6 % in the high-concentration spike treatment and 77.3 ± 8.5 % in the constant high-concentration treatment. The relationship between cumulative concentration (CT product) of formic acid and mite mortality in the 12 hives that were monitored for formic acid level was used to estimate

the CT₅₀ and CT₉₅ products for formic acid fumigation within the wintering building.

The CT₅₀ was 49.3 ppm*days and the CT₉₅ was 111.2 ppm*days (Fig. 3.3).

During fumigation, room temperature was 2.0 ± 0.2 °C (range -2.0 - 8.6 °C) in the high-concentration spike treatment room and 3.8 ± 0.1 °C (range -0.3 - 8.2 °C) in the constant high-concentration treatment room. Absolute humidity was 1.6 ± 0.02 g/m³ (range 1.1 - 2.3 g/m³) in the high-concentration spike treatment room and 4.3 ± 0.01 g/m³ (range 3.2 - 6.0 g/m³) in the constant high-concentration treatment room.

Experiment 2

The large difference between the formic acid concentration in the room air and hive air and the level of in-hive variability in formic acid concentration within hives was similar to that found in experiment 1 (Fig. 3.4). In the variable-ventilation fumigated room, the mean formic acid concentration in room air was 27.1 ± 7.7 ppm (range 4.7 - 54.1 ppm; Fig. 3.4) and approximately 5.6 L of 85 % formic acid were used. In the minimum-ventilation fumigated room, formic acid concentration in room air averaged 56.9 ± 8.1 ppm (range 11.4 - 76.0 ppm; Fig. 3.4) and approximately 6.0 L of 85 % formic acid were used. Acid concentrations in hive air averaged 6.3 ± 0.8 ppm (range 0 - 33.2 ppm) and 14.1 ± 2.2 ppm (range 0 - 59.0 ppm) in the variable-ventilation fumigated room and in the minimum-ventilation fumigated room, respectively. The cumulative concentration (CT product) that each hive received was negatively correlated with colony cluster size as measured in spring ($r = -0.39$, $P = 0.01$), but not correlated with final hive weight ($r = -0.05$, $P > 0.05$). The CT product in the hive also varied with row height ($F = 3.61$, $df = 4, 36$, $P = 0.01$) with the hives in row 5 having significantly higher formic acid concentration measurements than those in the bottom three rows (Fig. 3.5).

Mean abundance of varroa mites was affected by formic acid fumigation treatment as indicated by a significant formic acid treatment x time interaction ($F = 8.79$, $df = 1, 159$, $P < 0.01$; Fig. 3.6). In contrast, ventilation level did not affect the mean abundance of varroa mites as indicated by a non-significant ventilation treatment x time interaction ($F = 0.46$, $df = 1, 159$, $P > 0.05$; Fig. 3.6).

Neither acid fumigation ($F = 1.77$, $df = 1, 167$, $P > 0.05$) nor ventilation level ($F = 0.10$, $df = 1, 167$, $P > 0.05$) affected the honey bee cluster size over time as indicated by non-significant treatment x time interactions (before, mean 2.0 ± 0.2 , range 0.5 - 9.0; after, mean 4.9 ± 0.4 , range 0.0 - 10.0 frames of bees). Similarly, hive weight change over time was not affected by formic acid fumigation ($F = 0.00$, $df = 1, 167$, $P > 0.05$) or ventilation level ($F = 0.30$, $df = 1, 167$, $P > 0.05$) as indicated by non-significant treatment x time interactions (before, mean 35.4 ± 0.4 , range 27.2 - 42.7; after, mean 27.3 ± 0.5 , range 18.4 - 36.9 kg).

Queen drop during fumigation was significantly higher in the minimum-ventilation fumigated room (7 of 21 queens dropped) than in any other treatment room (0 of 21 queens dropped in each room) (Log-Rank, $P < 0.01$; Wilcoxon, $P < 0.01$; Fig. 3.7) and occurred throughout the fumigation period. The status of all colonies was checked on 17 April, revealing that an additional three colonies were queenless in the minimum-ventilation fumigated room, one in the variable-ventilation fumigated room, and one in the variable-ventilation unfumigated room. One of the colonies in the minimum-ventilation fumigated room that had dropped a queen was queenright on 17 April indicating that this colony had more than one queen at the start of the experiment.

Within fumigated rooms, the level of the maximum formic acid concentration found within a hive was marginally associated with the level of queen mortality ($P = 0.056$), but the cumulative concentration (CT product) as measured by in-hive CT product was not ($P > 0.05$). Significantly more queens were killed in colonies that had maximum formic acid concentrations of over 20 ppm (four of nine queens) than in those that received a maximum concentration below 9 ppm (three of 27 queens; $P = 0.050$). Queen mortality in colonies that received a maximum formic acid concentration of 10 - 19 ppm (zero of six queens) was not significantly different from those that received 0 - 9 ppm ($P > 0.05$) or over 20 ppm ($P > 0.05$). Three of 25 queens were killed in colonies that received a CT product of < 50 ppm*days, one of five with a CT product of 50 - 99 ppm*days, one of four with a CT product of 100 - 149 ppm*days, and two of five with a CT product of ≥ 150 ppm*days.

During fumigation, room temperature was 2.1 ± 0.1 °C (range -0.6 - 5.8 °C) in the variable-ventilation unfumigated room, 3.0 ± 0.01 °C (range 2.0 - 5.4 °C) in the variable-ventilation fumigated room, 2.2 ± 0.01 °C (range 0.7 - 7.0 °C) in the minimum-ventilation unfumigated room, and 4.4 ± 0.2 °C (range 1.2 - 11.0 °C) in the minimum-ventilation fumigated room. The absolute humidity was 1.6 ± 0.01 g/m³ (range 1.3 - 2.6 g/m³) in the variable-ventilation unfumigated room, 2.6 ± 0.03 g/m³ (range 1.6 - 4.4 g/m³) in the variable-ventilation fumigated room, 1.7 ± 0.02 g/m³ (range 1.3 - 2.7 g/m³) in the minimum-ventilation unfumigated room, and 4.3 ± 0.06 g/m³ (range 2.1 - 7.0 g/m³) in the minimum-ventilation fumigated room.

DISCUSSION

This study showed that it was possible to obtain in-hive concentrations of formic acid that brought the mean abundance of varroa mites below the economic threshold of 2 mites per 100 bees without killing queens under combinations of room temperature and ventilation that fall within the range used in normal commercial practice (Gatien and Currie, 2003). The cumulative concentration-mortality relationship based on in-hive concentrations of formic acid at ambient temperatures averaging 2 - 4 °C showed that the CT₅₀ and CT₉₅ for varroa mites were 49 ppm*days and 111 ppm*days, respectively. While the in-hive concentration of formic acid was quite variable, the data suggest that some of this variability might be reduced through minor improvements in ventilation and temperature control and/or better standardization of colonies subjected to fumigation.

Information about the exact timing of queen drop during fumigation with a high concentration of formic acid has not been reported previously. Observations of queen loss in outdoor trials with formic acid suggest that high ambient temperatures in combination with high concentrations of formic acid may contribute to queen loss (VonPosem, 1988). However, high ambient temperature alone is not the cause of queen loss as substantial mortality can occur when ambient temperatures are below 5 °C (Underwood and Currie, 2004; this study).

Experiment 1 was designed to determine the timing of queen loss under a sudden shock of formic acid as applied in the constant high-concentration treatment room in comparison with a more gradual increase in formic acid concentration as applied in the high-concentration spike treatment room. No queen loss was experienced in either treatment regime despite formic acid concentrations of up to 76 ppm in room air. Room

temperature during this fumigation was no more than 8.6 °C and in-hive formic acid concentrations did not exceed 18 ppm. Underwood and Currie (2004) found excessive queen loss in treatments where the average room concentration averaged only 41 ppm, but in-hive concentrations averaged 17 - 25 ppm and maximum concentrations of 38 ppm were observed in some colonies. The reason for less queen loss under higher acid concentrations in room air is not known, but may be related to room temperature during fumigation.

Experiment 2 tested the hypothesis that using ventilation to control room temperature and formic acid concentration during fumigation might prevent queen loss during fumigation even if relatively high concentrations of formic acid are present in room air. The results showed that substantial queen loss occurred only in the minimum-ventilation fumigated room, which had a mean in-hive formic acid concentration of 14 ppm (maximum 59 ppm). This room had the highest mean temperature (4.4 °C) during fumigation with a maximum temperature of 11 °C. No queen loss was experienced when room temperature was regulated by ventilation during the high-concentration treatment (maximum temperature of 8 °C) where in-hive formic acid concentration averaged only 6 ppm (maximum 33 ppm). In-hive formic acid concentration could have been lower in the variable-ventilation treatment room because of the lower temperature, because of the increased ventilation, or both. As formic acid is relatively inexpensive, increased ventilation may be a simple means for decreasing variability in in-hive formic acid concentration. Increased ventilation would increase the volume of acid required by only 8 %.

During experiment 2, queen loss occurred over an extended period of time, indicating that a sudden high concentration of formic acid in room air was not the cause of queen loss. In-hive formic acid concentration was more important. Queens that were lost tended to be from colonies with higher maximum in-hive formic acid concentrations, suggesting that there is an upper lethal formic acid concentration for queen honey bees fumigated at low temperatures. Queens exposed to higher cumulative concentrations (CT products) were not significantly more likely to die during fumigation than those exposed to lower cumulative concentrations. This suggests that queen loss is due to acute, not chronic exposure to formic acid. Thus, standardization and control of in-hive formic acid concentration among hives should help in the avoidance of lethal peak formic acid concentrations and subsequent queen loss.

During indoor winter fumigation for 48 h, Underwood and Currie (2004) found that the formic acid concentration in room air was significantly higher than in hive air. The results of this study confirm this and show that room and hive air do not reach equilibrium even after 7 days. Therefore, in practice, CT products will have to be based on in-hive formic acid concentrations rather than on room air concentrations.

Underwood and Currie (2004) found that an in-hive CT product of 37 ppm*days during a 48-hour period reduced the mean abundance of varroa mites to below the economic threshold. This study showed that an in-hive CT product of 111.2 ppm*days given over 5 days would be required to provide 95% control, but this level of control may not be required to decrease the mean abundance of varroa mites to an acceptable level. For example, in experiment 2, in-hive CT products of 44 and 99 ppm*days were achieved during the fumigation period for the variable- and minimum-ventilation treatments,

respectively. Although these cumulative concentrations were lower than the CT_{95} calculated in experiment 1, they brought the mean abundance of varroa mites to 0.2 mites per 100 bees. In addition, these CT products were obtained by pooling data from various hives and using the mite as the experimental unit. The value is our best estimate of the concentration-time combination necessary for good efficacy during treatment.

The position of the hive in the treatment room and the size of the honey bee cluster both influenced in-hive formic acid concentration during treatments. Previous work has shown that formic acid is evenly distributed throughout the treatment room during fumigation and that stacking colonies three high does not affect in-hive formic acid concentration (Underwood and Currie, 2004). The results of this study confirm this, but show higher concentrations in colonies in the fourth and fifth row than in colonies in the bottom three rows. Changes in ventilation level or air circulation within the room should easily rectify this. For example, variation in in-hive formic acid concentration was much higher in the minimum-ventilation fumigated room than in the variable-ventilation fumigated room. Thus, temperature and/or increased air mixing controlled by ventilation may help standardize the in-hive concentration of formic acid in hives stacked at different levels. Initial trials on indoor fumigation used minimum ventilation because it was thought that it might not be possible to obtain formic acid concentrations high enough for varroa mite control at high air exchange rates (Underwood and Currie, 2004). The current study suggests that this may not be a major concern.

Other factors may also affect in-hive formic acid concentration variability. The cumulative concentration (CT product) that each colony received had a weak negative correlation with cluster size, suggesting that rates of sorption of formic acid may vary

with cluster size. If producers could minimize variability in cluster size, it may help to standardize the CT product each colony receives and thus allow shorter fumigation periods. In addition, fumigating early in winter when cluster sizes are more similar might help.

Outdoor formic acid treatments in spring or fall have met with some success, but require repeated apiary visits and treatment of individual hives in an uncontrolled environment (e.g. Krämer, 1986; Bracey and Fischer, 1989; Clark, 1994; Feldlaufer et al., 1997; Daniels et al., 1999; Kochansky and Shimanuki, 1999; Hood and McCreadie, 2001; Skinner et al., 2001; Gaten and Currie, 2003; Ostermann and Currie, 2004). Indoor fumigation allows fumigation of many hives simultaneously and in one place at a time when very little brood is found in colonies. A single treatment in winter would allow the bees to go into the spring with a mean abundance of varroa mites that is below the economic threshold.

This study showed that indoor winter fumigation of honey bee colonies with an in-hive CT product of up to 141 ppm*days of formic acid at 2 - 4 °C can control varroa mites without harming queens under certain environmental conditions. In-hive variability in formic acid concentration appeared to be related in part to the level of hive stacking and honey bee cluster size. Lid type, tightness of fit, and compression may also play a role. Differences in concentration between room and hive air did not reach equilibrium even after 7 days of fumigation. Therefore, CT product calculations should be based upon hive air rather than room air. Because CT products change with concentration and temperature, environmental conditions need to be taken into account when fumigating with formic acid (Underwood and Currie, 2003). Use of temperature-

dependent room ventilation decreased variability in in-hive formic acid concentration and significantly reduced queen loss. Further improvements in air mixing within rooms and standardization of cluster sizes should improve the delivery of consistent concentrations to hives.

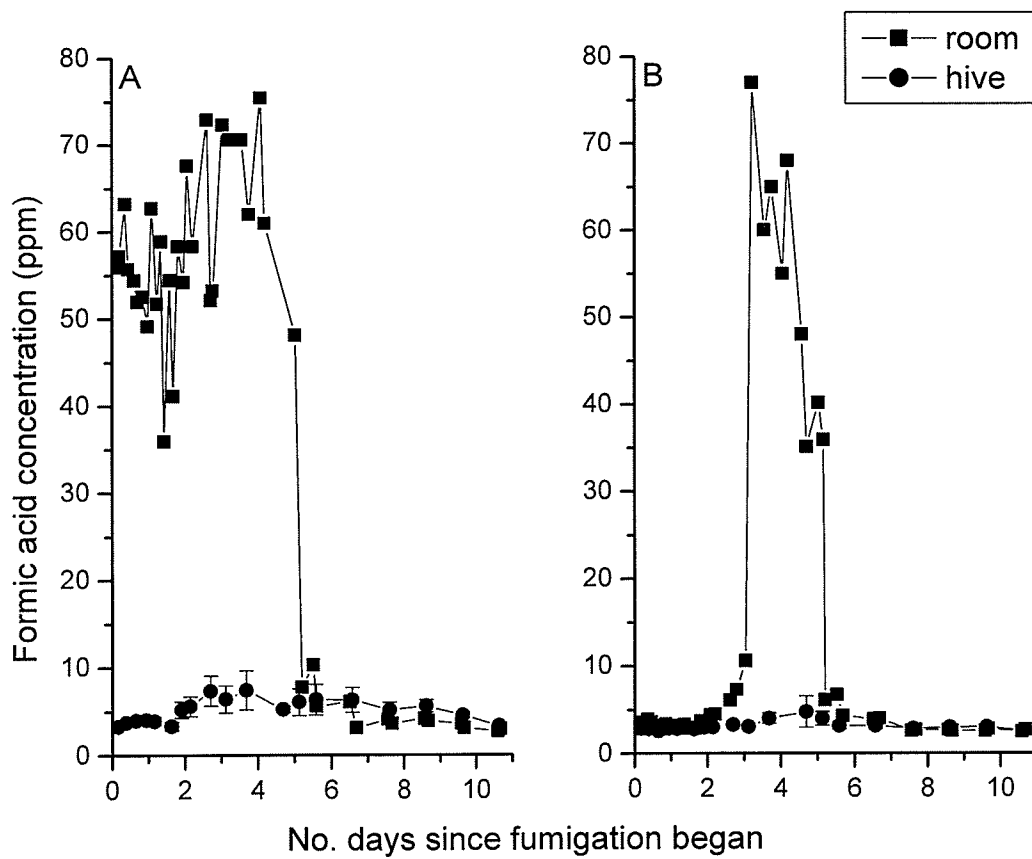


Figure 3.1. Mean (\pm SE) formic acid concentration in room and hive air in the constant high-concentration treatment (A) and the increasing concentration formic acid treatment (B) in experiment 1. Fumigation took place from 17 – 22 December 2001.

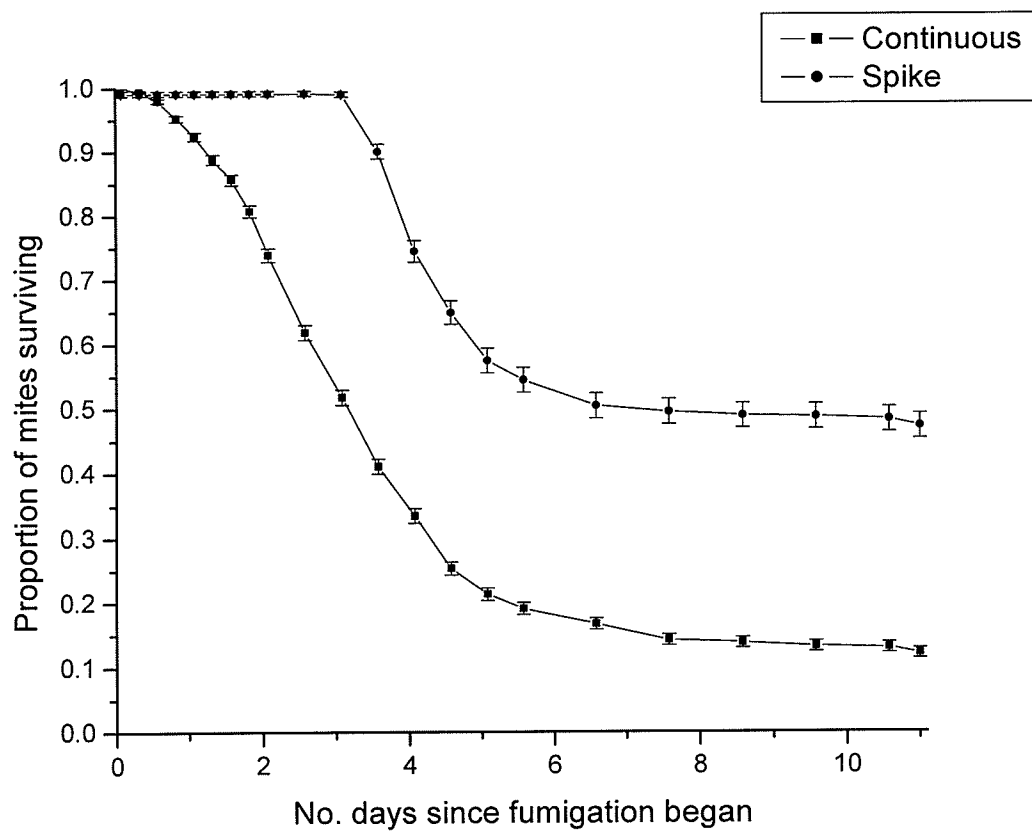


Figure 3.2. Varroa mite mortality (mean \pm SE) over time in colonies fumigated with formic acid for 5 days in experiment 1. $n = 15$ colonies per treatment.

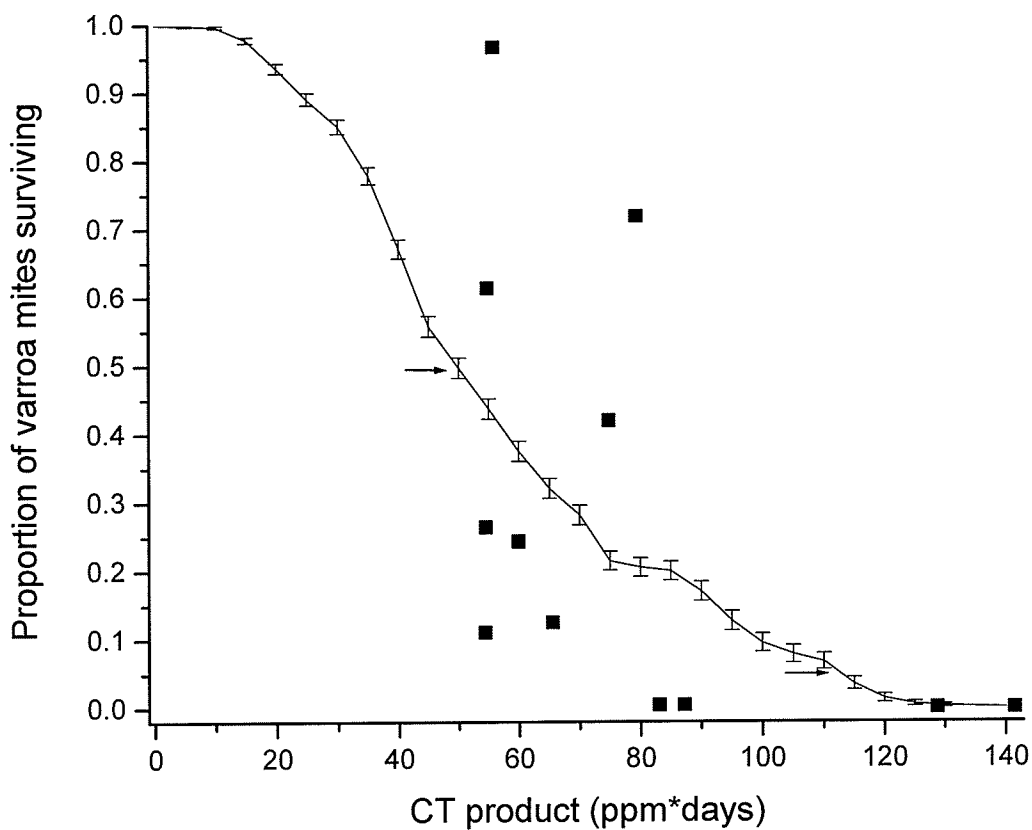


Figure 3.3. Concentration-response curve for varroa mites in colonies fumigated with formic acid for 5 days in experiment 1. The line represents the mean (\pm SE) cumulative mite mortality ($n = 1,107$ varroa mites) as each CT product was achieved. The filled squares (■) represent the total proportion of mites surviving in each hive ($n = 1$ hive for each point) at the final CT product for that hive. The horizontal arrows indicate the point on the line where the CT_{50} and CT_{95} are reached.

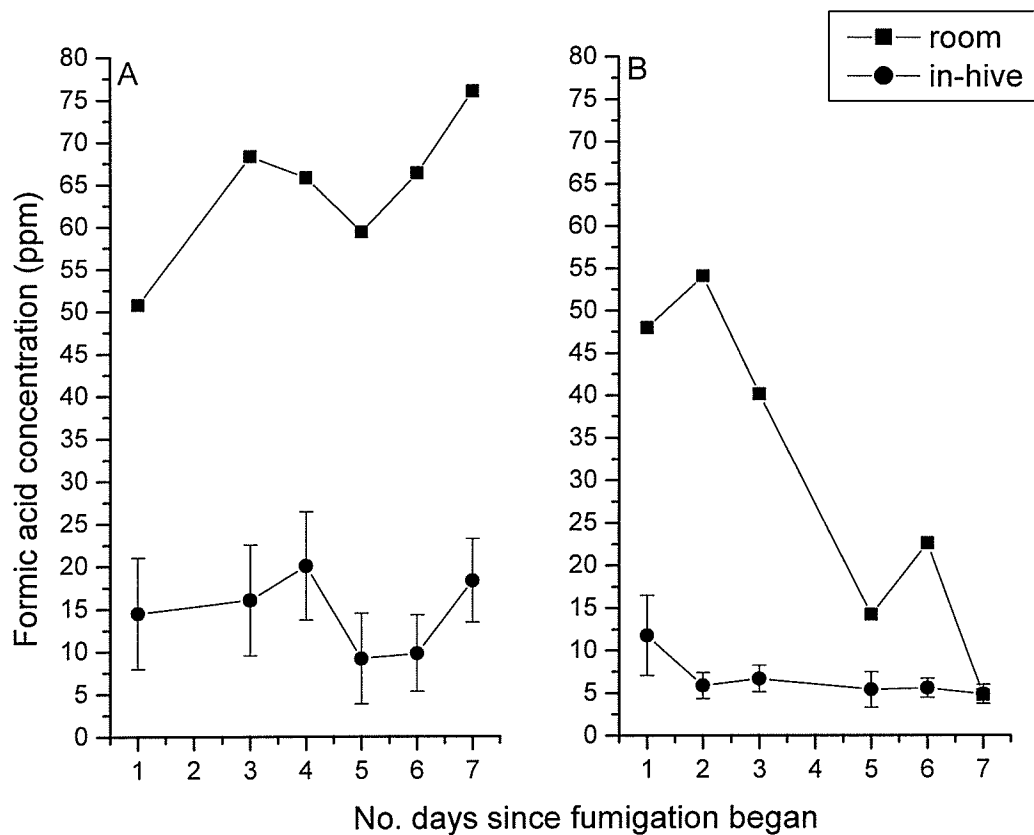


Figure 3.4. Formic acid concentration in the minimum-ventilation fumigated room (A) and variable-ventilation fumigated room (B) in experiment 2. Filled squares (-■-) represent measurements taken in the center of each treatment room ($n = 1$) while the filled circles (-●-) represent measurements from in hives (mean \pm SE, $n = 21$). Fumigation took place from 4 - 11 March 2002.

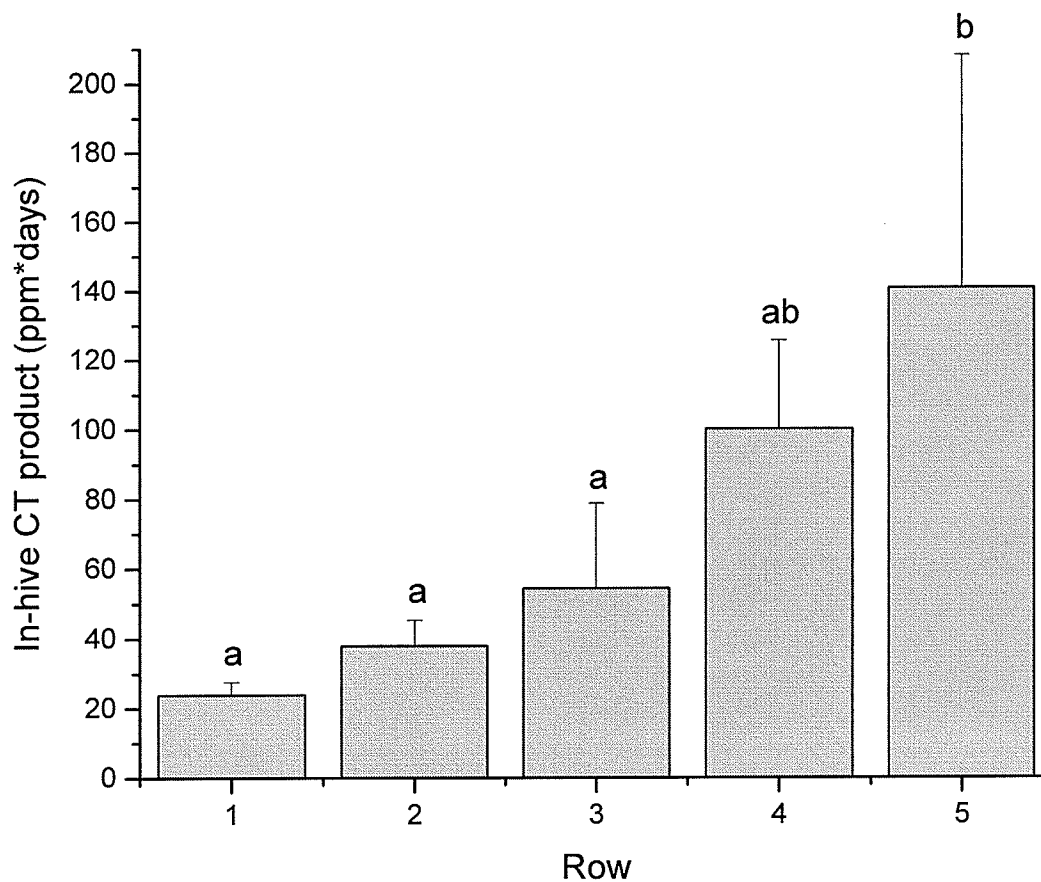


Figure 3.5. Mean in-hive CT product of formic acid (\pm SE) in hives stacked in acid

treatment rooms from the floor (row 1) to the top of the stack (row 5) over the 7 days of fumigation in experiment 2. For rows 1 – 4 $n = 10$ hives each, while for row five $n = 2$ hives. Bars with different letters are significantly different from each other (SNK; $P < 0.05$).

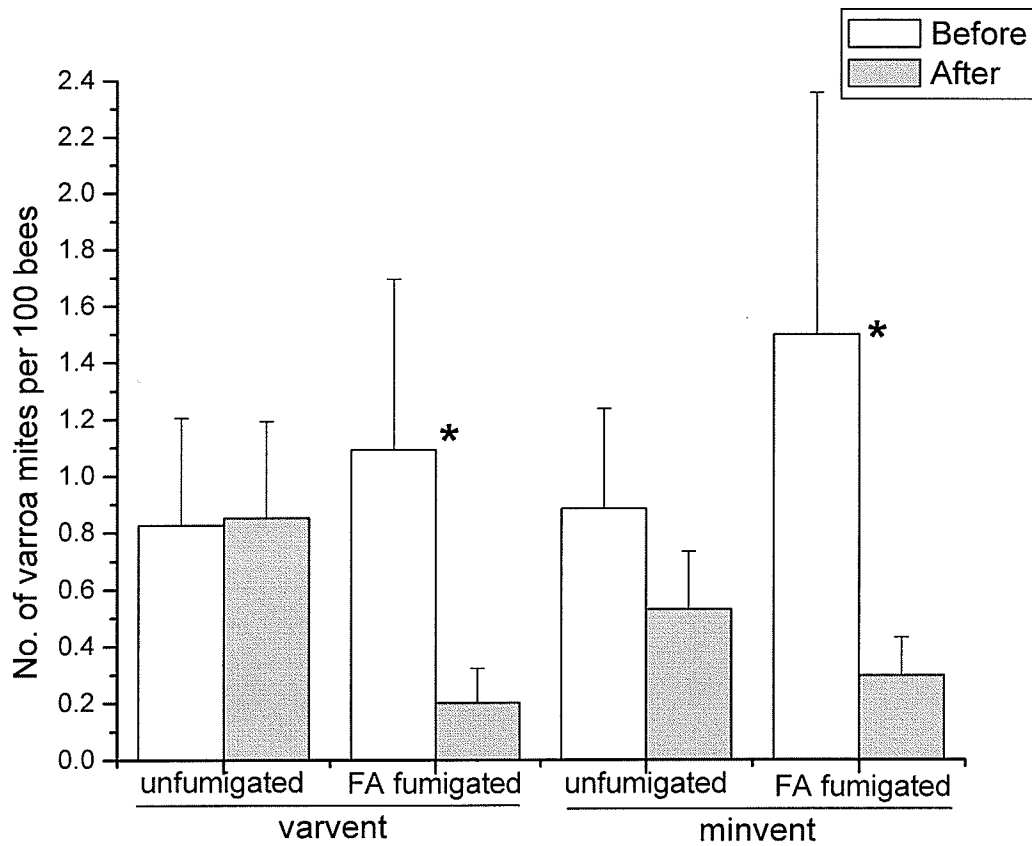


Figure 3.6. Varroa mite mean abundance (\pm SE) measured before and after wintering in colonies that were unfumigated with minimum ventilation (minvent) ($n = 21$ before, $n = 20$ after) or variable ventilation (varvent) ($n = 21$ before, $n = 19$ after) or treated with a high concentration of formic acid (FA fumigated) with minimum ventilation ($n = 21$ before, $n = 19$ after) or variable ventilation ($n = 21$ before, $n = 18$ after) for 7 days. An asterisk indicates a significant treatment x time interaction within acid or ventilation levels ($P < 0.05$).

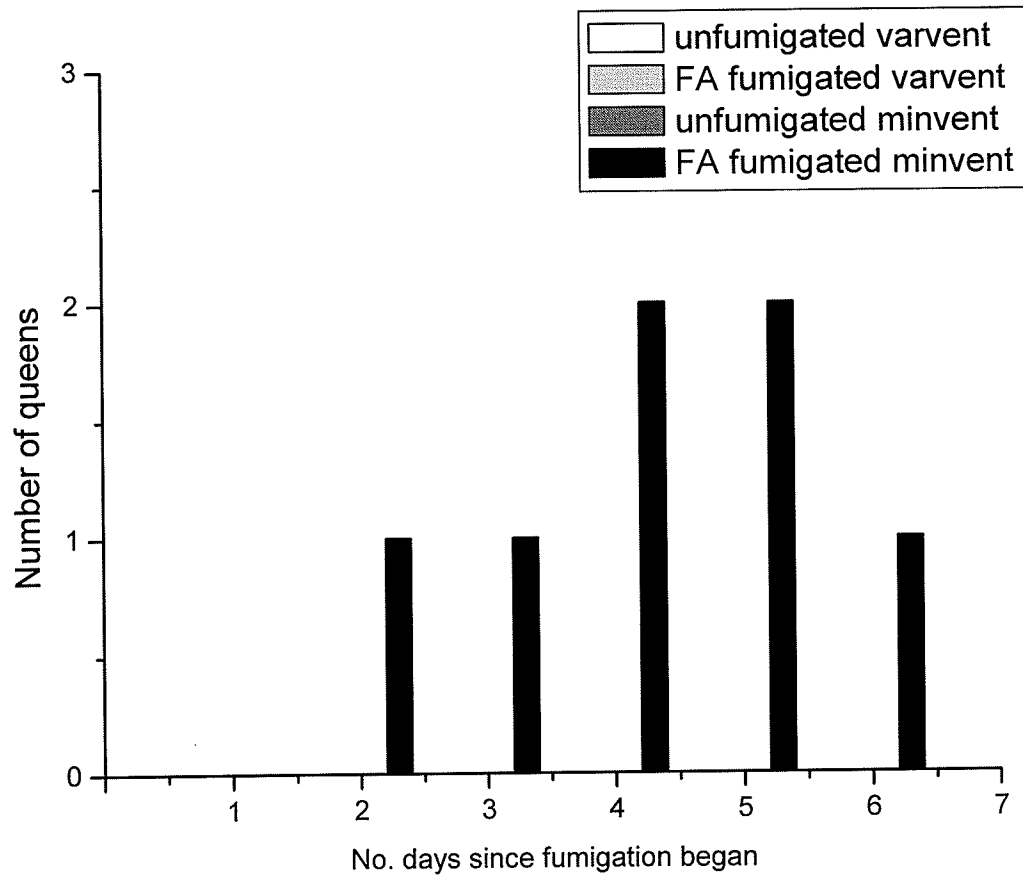


Figure 3.7. Timing of queen drop onto the white board in colonies that were unfumigated with minimum ventilation (minvent) or variable ventilation (varvent) or treated with a high concentration of formic acid (FA fumigated) with minimum ventilation or variable ventilation for 7 days ($n = 21$ queens for each treatment). Fumigation took place from 4 - 11 March 2002.

CHAPTER 4. WHAT IS THE MOST EFFICACIOUS CONCENTRATION X TIME COMBINATION DURING INDOOR WINTER FUMIGATION WITH FORMIC ACID FOR MAXIMIZING VARROA MITE CONTROL WHILE MINIMIZING THE EFFECT ON WORKER AND QUEEN HONEY BEES?

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 ectoparasitic varroa mite, *Varroa destructor* Anderson and Trueman, determines the efficacy of the treatment. Because high concentrations of formic acid can cause queen mortality, we hypothesized that a high CT product given as a low concentration over a long period rather than as a high concentration over a short period would allow effective control of varroa mites without the detrimental effects on queens. The objective of this study was to assess different concentration-time combinations with similar cumulative concentrations (CT products) of formic acid in controlling varroa mites while minimizing the effect on worker and queen honey bees.

Starting on 11 January 2001, treated colonies were exposed to a low, medium, or high concentration of formic acid until a CT product of approximately 390 ppm*days in room air was realized. The treatments consisted of a long-term low concentration of 19 ppm for 27 days, a medium-term medium concentration of 39 ppm for 10 days, a short-term high concentration of 45 ppm for 9 days, and an untreated control. All three acid treatments caused significant daily varroa mite mortality that was reflected in a significant decrease in the mean abundance of mites as compared to the control. This

trend differed slightly in colonies from two different beekeepers. Daily worker mortality was significantly increased by the short-term high concentration treatment but this was not reflected in an increase in colony mortality or a substantial decrease in the size of the worker population. Queen mortality was significantly greater under the medium-term medium concentration and the short-term high concentration treatments than in controls.

INTRODUCTION

Fumigating honey bee, *Apis mellifera* L., colonies with formic acid is a feasible option to control varroa mites, *Varroa destructor* Anderson and Trueman, and other pests. Formic acid can be applied in the field during the spring or fall (e.g. 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, chapter 3). Indoor fumigation looks promising and has the advantages of control over ambient temperature and added susceptibility of mites due to a lack of honey bee brood (Underwood and Currie, 2003, 2004, chapter 3).

Standardization of fumigation methods for reliable pest control requires the determination of the most effective concentration-time combination of the fumigant, 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 time can be expressed as a value known as the concentration x time (CT) product, expressed as the CT_{50} or CT_{95} product for values causing 50 or 95 % mortality, respectively (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 concentrations and temperatures. This value was then used as a

basis for tests of entire colonies in a wintering building (Underwood and Currie, 2004, chapter 3). A CT product of 37 ppm*days in hive air (82 ppm*days in room air) given over 2 days is effective at killing varroa mites, but also causes significant queen loss (Underwood and Currie, 2004) and a CT product of 70 ppm*days (114 ppm*days in room air) given over 5 days had a similar effect (chapter 3). 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 (chapter 3). 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 period of 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 a similar CT product over three time periods to determine the efficacy of each concentration-time combination in controlling varroa mites while minimizing the effect of treatment on worker and queen honey bees.

MATERIALS AND METHODS

Prior to 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 the late fall of 2001. Pre-treatment 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 using an alcohol wash method with a mechanical shaker

(Gatien and Currie, 2003). 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, colonies were weighed and brought indoors where they were positioned in stacks placed randomly in a large holding room (5.2 x 7.2 x 2.5 m) prior to the start of the experiment. Each hive had a completely open bottom entrance (37 x 2.5 cm) and a small top entrance (AF; 1.9 x .7 cm; UM; 5.7 x 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. The white boards collected worker and queen bees and varroa mites falling from the honey bee cluster and allowed them to be removed and sampled easily. White board contents were collected for a 7-day pre-treatment period and daily for 36 days during and immediately following fumigation. On 15 February, stacks of hives were again placed randomly in a large holding room where white board contents were collected biweekly until colonies were moved outdoors.

Fumigation

Fumigation tests were conducted in four 3.0 x 1.7 x 2.5 m treatment rooms in the wintering building at the University of Manitoba in Winnipeg, Manitoba, Canada (N 49° 48' 32", W 97° 07' 37"). Each room simulated the air flow conditions of a standard

commercial wintering building with a fan jet air distribution system (Gruszka, 1998), but was modified to allow formic acid fumigation (see Underwood and Currie, 2004 for details). 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 x 25.0 x 5.5 cm) of liquid containing three T-shaped humidifier plates (11.5 x 16.7 x 0.2 cm, 64-3119-0, Dundas-Jafine Industries Ltd., Weston, Ontario, Canada) 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 L/s/m³. Before and after fumigation, temperature and ventilation in all rooms was controlled with three additional step-wise 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 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 L of 25 % formic acid, a humidifier sponge (30 x 19 x 5 cm, Air-King Ltd., Brampton, Ontario, Canada), and a reservoir bag with 3 L of 65 % formic acid. The pan in the medium-term medium-

concentration treatment started with 1 L of 40 % formic acid, three T-shaped humidifier plates (11.5 x 16.7 x 0.2 cm, 64-3119-0, Dundas-Jafine Industried Ltd., Weston, Ontario, Canada), and a reservoir bag with 3 L of 65 % formic acid. The pan in the short-term high-concentration treatment started with 1 L of 65 % formic acid, three T-shaped humidifer plates, and a reservoir bag with 3 L 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 L in the long-term low-concentration treatment room, 8.0 L in the medium-term medium-concentration treatment room and 7.6 L 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 concentration x time (CT) product of approximately 390 ppm*days (based on room air) was realized in each experimental room. 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*days 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 days (worker mortality was evident on the 10th 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 days (439 ppm*days) and the long-term low-concentration treatment was 18.9 ± 1.1 ppm formic acid for 27 days (492 ppm*days). 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) calibrated using Dräger tubes (Tube No. 6722101 with Accuro 9022003 gas detector pump, National Dräger, Pittsburgh, PA) or by using Dräger tubes directly. 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 Scientific Limited, 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, 5-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 three hives within each room was sampled daily during fumigation and after fumigation until the concentration dropped below 1 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 minutes throughout the experiment starting one week prior to fumigation.

To collect varroa mite and bee samples within the rooms during fumigation, a low pressure ambient air breathing apparatus (Model 1023-P152G-G608X, Gast Manufacturing Corp., Benton Harbor, MI, USA) with continuous flow supplied air hood (Model R799, Wilson Safety Products, Reading, PA, USA) was used. This allowed the

experimenter to enter the experimental rooms safely during fumigation at the relatively low concentrations of formic acid used in our experiments.

Following fumigation

The bees were moved outside on 8 April 2002. 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. The value for the mean abundance of varroa mites at the start of the experiment was calculated by dividing the number of mites present at the start of fumigation by the number of bees present at the start of fumigation, which was the total number of bees that were collected in white board samples during and after fumigation plus the final size of the honey bee population as estimated on 8 April. Proportional mite mortality values were then obtained by dividing the cumulative daily mite mortality to day i by the total number of mites that dropped during the entire trial.

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, the size of the honey bee brood population was estimated. The brood area (cm^2), of both

capped and uncapped brood, was estimated using a piece of plexiglass 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 Inc., 1999). Treatment room and sample height were the main plot factors and time was the subplot factor. Treatment room x sample height was the error term for the main plot factors. When significant treatment room differences were found ($P < 0.05$), a Student-Neumann-Keuls (SNK) multiple range test was used to compare differences between means (SAS Institute Inc., 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 time. Treatment room x beekeeper x 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 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 Inc., 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 using a repeated measures ANOVA with a heterogeneous autoregressive covariance structure (PROC MIXED; SAS Institute Inc., 1999). Mean abundance of varroa mite data were arcsine transformed while worker bee population estimates were log transformed prior to analysis. When significant interactions between treatment room and 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 alpha values.

The effect of treatment room on queen and colony survival was analyzed by using Fisher's exact test and comparing each treatment to the control (SAS Institute Inc., 1999). The effect of treatment room on pre-treatment 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 using an ANOVA. The influence of beekeeper on the initial weight and cluster size of colonies was analyzed as a completely randomized design using an ANOVA.

RESULTS

Formic acid concentration

When all three acid-treated rooms were being fumigated simultaneously (during the first 9 days 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 ($F = 76.71$, $df = 2, 27$, $P < 0.001$; SNK; Fig. 4.1). During the 27 days of fumigation in the long-term low concentration treatment room, formic acid concentration in room air averaged 17.8 ± 1.0 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 days 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 days of fumigation in the short-term high concentration treatment room, formic acid concentration in room air averaged 52.4 ± 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

Prior to 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 x beekeeper x 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 x time interactions (AF; $F = 10.31$, $df = 123, 780$, $P < 0.0001$; UM; $F = 20.71$, $df = 123, 984$, $P < 0.0001$; Fig. 4.2). 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. 4.2).

Worker bee daily mortality

Prior to 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 x

beekeeper x time interaction for cumulative worker bee mortality ($F = 0.17$, $df = 123$, 534 , $P > 0.05$), however, there was a significant treatment room x time interaction ($F = 11.37$, $df = 123$, 534 , $P < 0.0001$; Fig. 4.3). Bee mortality was not significantly different between treatment rooms until after day 10, when bee mortality in the short-term high-concentration treatment was significantly higher than the control and in all other treatments (Duncan's; Fig. 4.3). From day 11 until day 83, bee mortality rates in all treatment rooms were fairly consistent (Fig. 4.3). There was no treatment room x beekeeper effect ($F = 0.92$, $df = 3$, 12 , $P > 0.05$).

Queen bee and colony mortality

The proportion of queens killed during fumigation differed between treatment rooms ($P < 0.001$) with zero of 20 queens dropping out of the cluster and onto the whiteboard in the control room, zero of 21 dropping in the long-term low-concentration treatment, five of 21 queens dropping in the medium-term medium-concentration treatment, and seven of 21 queens dropping in the short-term high-concentration treatment (Fig. 4.4). Significantly more queens dropped in the short-term high concentration ($P < 0.01$) and medium-term medium concentration ($P < 0.05$) than in the control. Two queens dropped in the control room; one prior to 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 days 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. 4.4). Queenless colonies were successfully requeened in the spring following 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 x time interaction ($F = 11.36$, $df = 3, 66$, $P < 0.0001$; Fig. 4.5). All three acid treatments reduced the mean abundance of varroa mites relative to untreated controls.

Honey bee population size

At the start of fumigation, colonies from beekeeper AF had significantly larger populations than those from UM (AF; 15608 ± 1239 bees; UM; 11017 ± 1174 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 x time interaction ($F = 12.11$, $df = 3, 66$, $P < 0.001$; Fig. 4.6). 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 cm²; $F = 0.87$, $df = 3, 30$, $P > 0.05$), capped brood area (mean 39 ± 6 cm²; $F = 0.73$, $df = 3, 30$, $P > 0.05$), or the uncapped brood area (mean 34 ± 8 cm²; $F = 0.56$, $df = 3, 30$, $P > 0.05$) of surviving colonies.

Hive weight

Treatment room did not affect hive weight loss as indicated by a non-significant treatment room x time interaction ($F = 0.32$, $df = 3, 60$, $P > 0.05$). Before entering the wintering building, hives averaged 36.2 ± 0.5 kg, while 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 figure 4.7. Prior to fumigation, there was no apparent difference in room temperature. However, during the first 9 days 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 day sampling period, the control room temperature ranged from 1.2 to 11.0 °C, while the absolute humidity averaged $2.4 \pm 0.0 \text{ g/m}^3$ (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 $1.9 \pm 0.0 \text{ g/m}^3$ (range 1.4 – 2.9 g/m^3). During the 10 days of fumigation in the medium-term medium-concentration treatment, the temperature ranged from 7.8 to 17.9 °C, while the absolute humidity averaged $5.9 \pm 0.0 \text{ g/m}^3$ (range 3.7 – 7.4 g/m^3). During the 9 days of fumigation in the short-term high-concentration treatment, the temperature ranged from 5.8 to 14.9 °C, while the absolute humidity averaged $7.0 \pm 0.1 \text{ g/m}^3$ (range 3.7 – 10.5 g/m^3).

DISCUSSION

Indoor winter fumigation with a CT product of 390 ppm*days of formic acid in room air significantly increased varroa mite mortality at all three treatment durations. However, when this CT product was reached in 9 or 10 days it was detrimental to worker bee survival, queen bee survival, or both. The best concentration-time combination 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), while mites in colonies from UM were susceptible (Underwood and Currie, 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 previously reported. In addition, AF colonies were heavier at the start of the experiment and had larger bee populations than those from UM. Underwood and Currie (chapter 3) showed that cluster size, but not hive weight, was negatively correlated with in-hive CT product during formic acid fumigation and that mite mortality was 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 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 44.5 ± 10.5 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 dropped out onto the white board as a result of fumigation, respectively. In contrast, despite the realization of a relatively higher CT product in the room air in the long-term low-concentration treatment, no queens were found in white board samples. The queens that dropped 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 two days 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 and Currie (chapter 3) determined that queen loss was associated with peak formic acid concentrations above 20 ppm in the hive. Queen losses in this experiment occurred in the final 2 days 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 may also 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;

chapter 3). The ventilation rate in experimental rooms in this study was held constant at 0.4 L/s/m^3 to maintain consistent formic acid levels in the room air. Therefore, room temperatures averaged $7.5 - 11.5^\circ\text{C}$ 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). Chapter 3 showed that allowing ventilation rates to increase when room temperature exceeds 6°C could drastically reduce queen loss even when high concentrations of formic acid were 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, chapter 3). 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 (chapter 3). 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 were effective in killing varroa mites, with averages of 93 and 83 % mortality, respectively, but 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 did not increase worker or queen bee mortality.

Efficacy varied with beekeeper within each treatment room indicating that colony state and/or the source of varroa mites may affect fumigation results and should be considered when determining the appropriate concentration x time combination required.

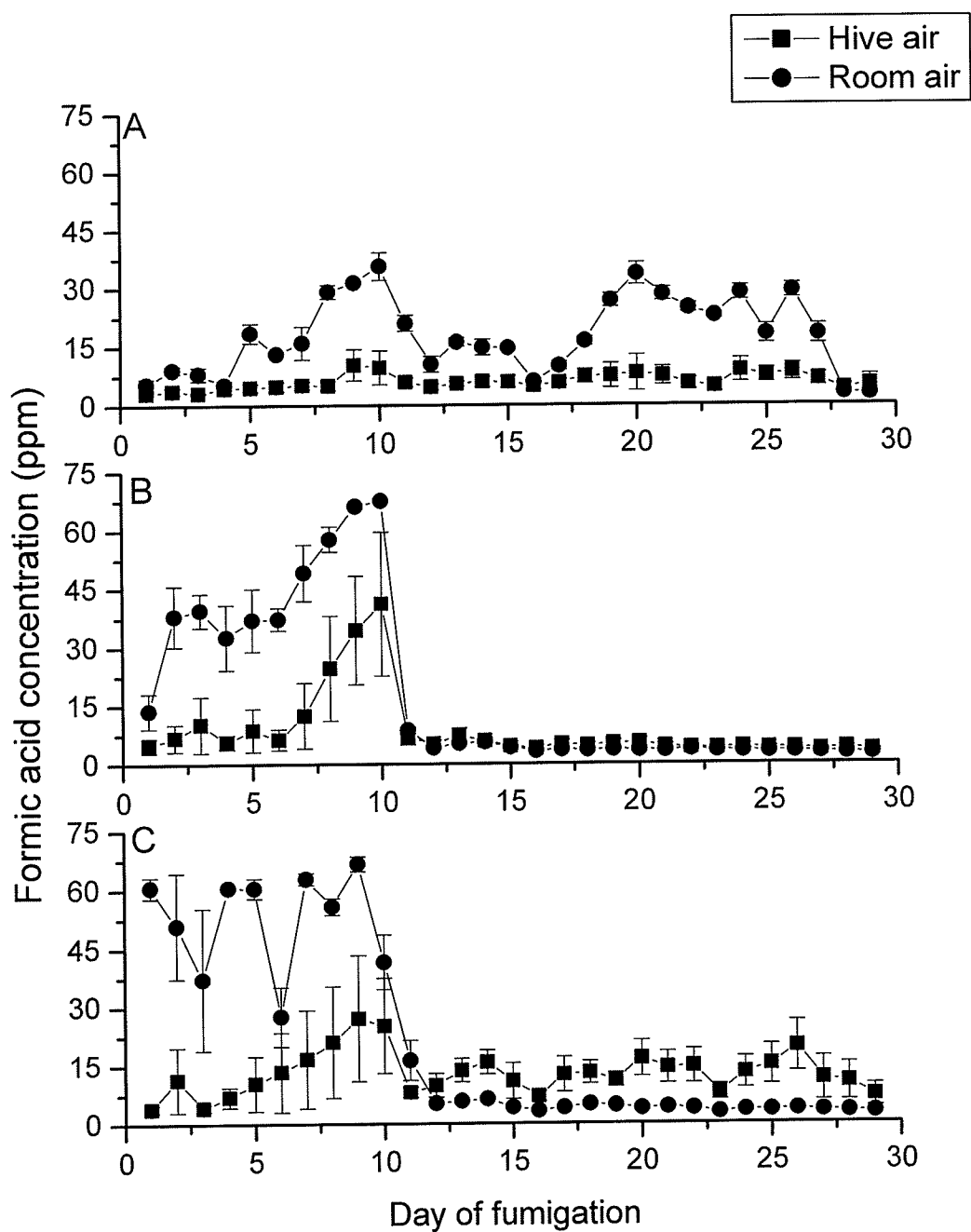


Figure 4.1. Concentration of formic acid (mean \pm SE) in room and hive air during fumigation in rooms receiving a low concentration for 27 days (A), a medium

concentration for 10 days (B) or a high concentration for 9 days (C). $n = 3$ for each point.

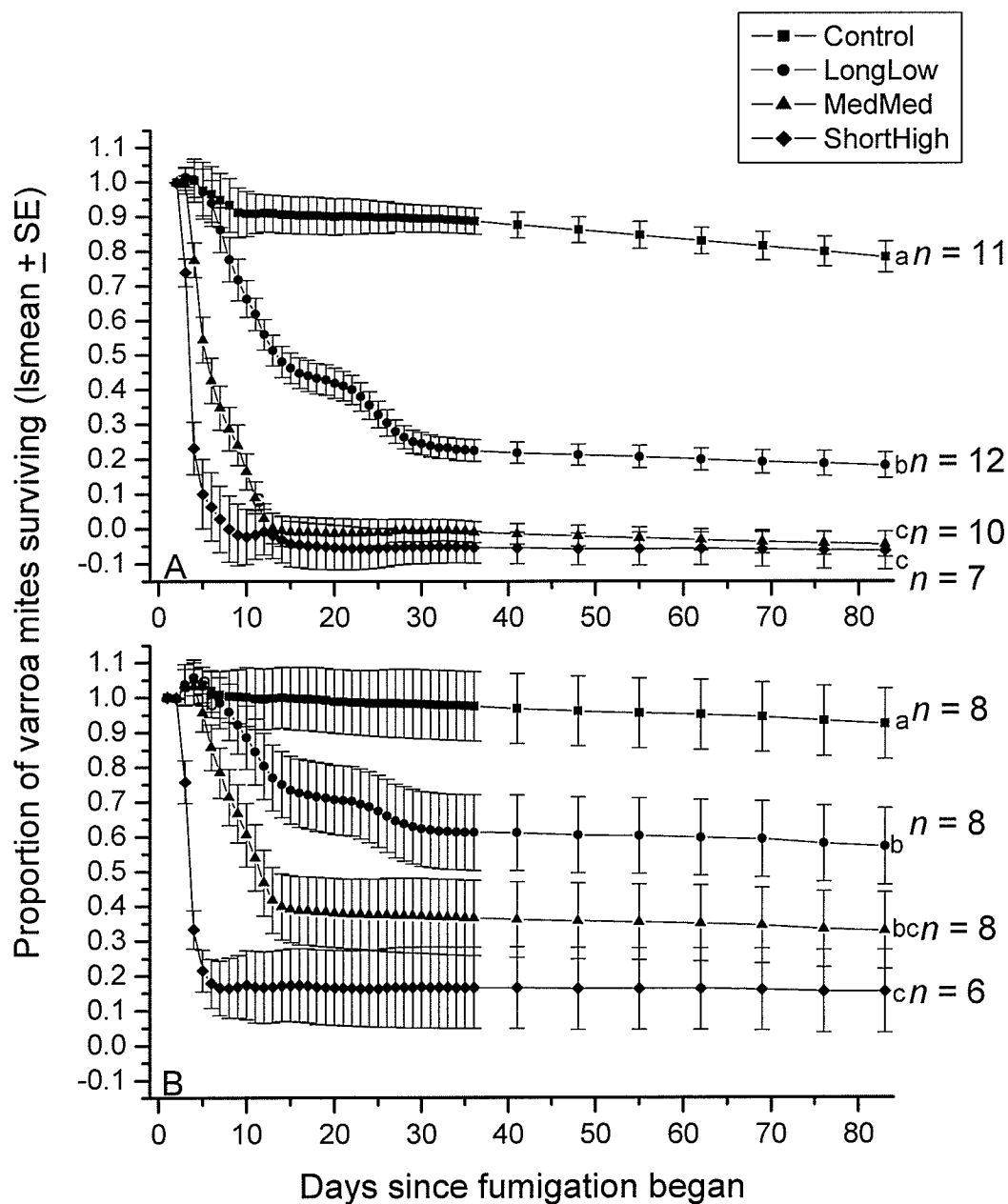


Figure 4.2. Mean proportion of varroa mites (\pm SE) surviving over the course of the experiment in colonies from beekeeper UM (A) or AF (B) in rooms that were untreated (Control), or received a long-term low concentration (LongLow), a medium-term medium concentration (MedMed) or a short-term high

concentration (ShortHigh) of formic acid. Means followed by the same letter are not significantly different from each other (Duncan's; $P > 0.05$). n = number of hives. UM colonies contained an average of 347 ± 51 varroa mites, while AF colonies contained an average of 701 ± 133 varroa mites.

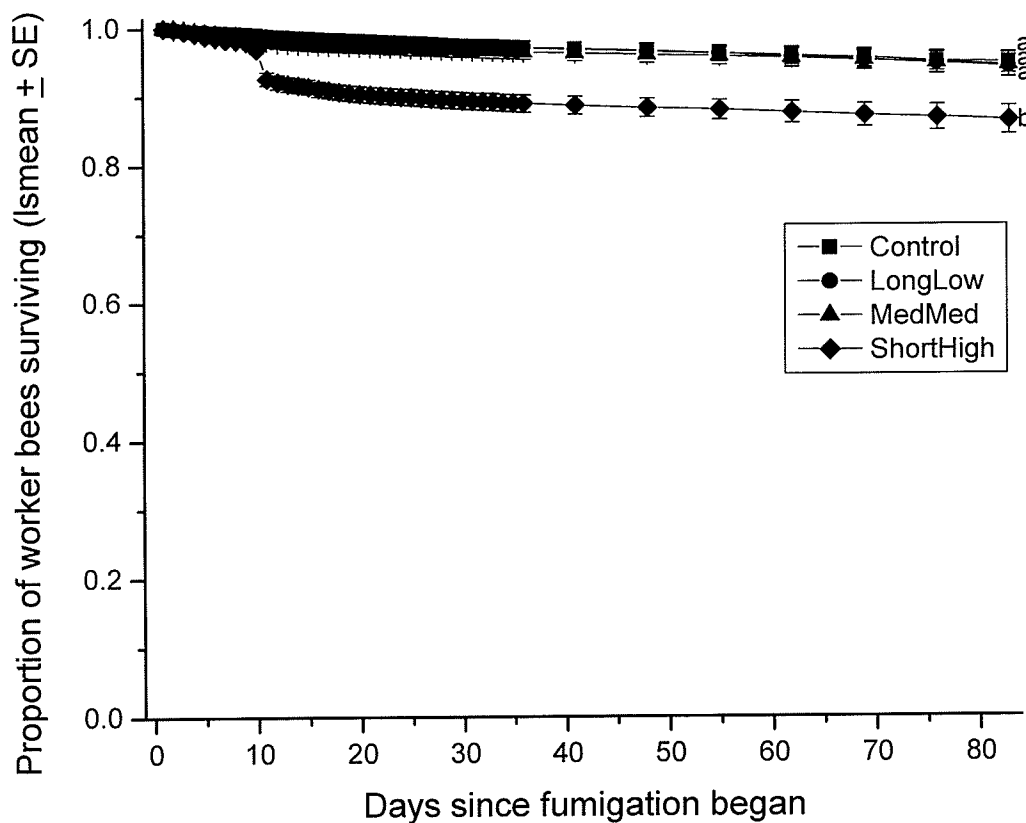


Figure 4.3. Mean proportion of worker bees (\pm SE) surviving over the course of the experiment in rooms that were untreated (Control; $n = 19$ hives), or received a long-term low concentration (LongLow; $n = 20$ hives), a medium-term medium concentration (MedMed; $n = 18$ hives) or a short-term high concentration (ShortHigh; $n = 13$ hives) of formic acid. Means followed by the same letter are not significantly different from each other (Duncan's; $P > 0.05$).

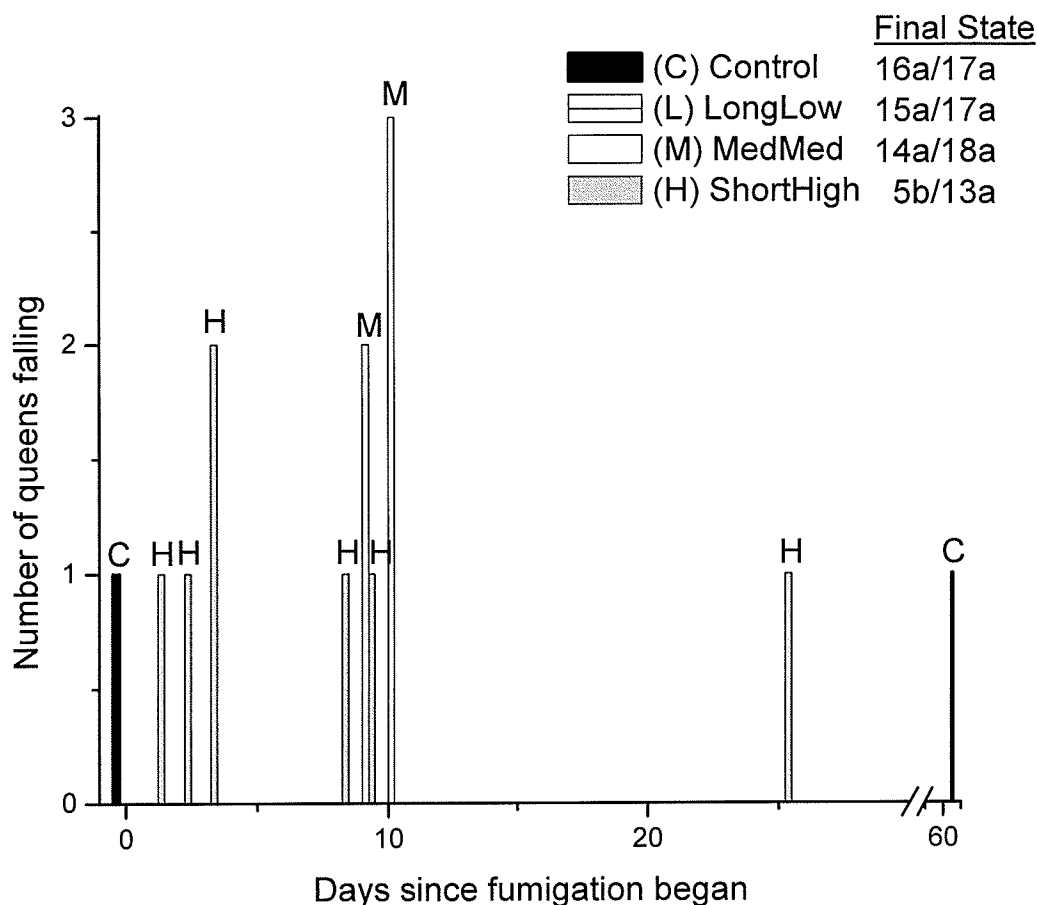


Figure 4.4. Number of queens dropping onto white boards over the course of the experiment in rooms that were untreated (Control), or received a long-term low concentration (LongLow), a medium-term medium concentration (MedMed) or a short-term high concentration (ShortHigh) of formic acid. $n = 21$ colonies 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 (Fisher's exact test; $P < 0.05$).

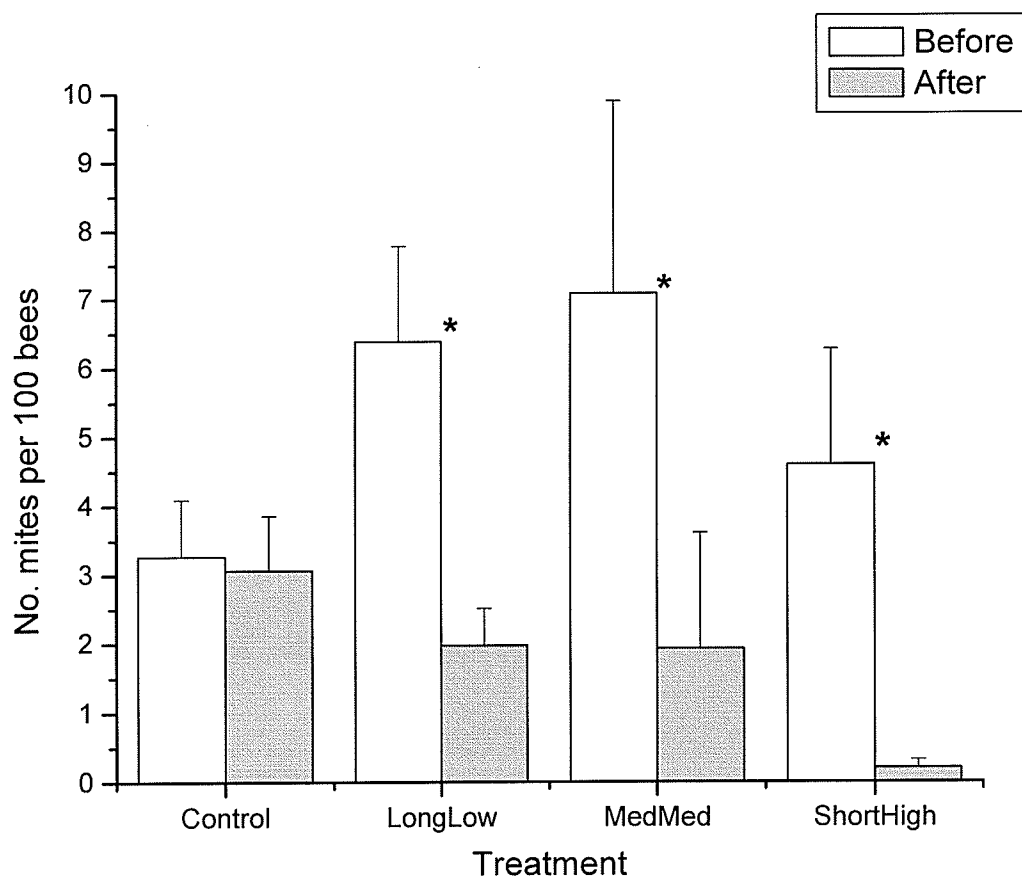


Figure 4.5. Mean abundance of varroa mites (\pm SE) measured before and after wintering in colonies that were untreated (Control; $n = 19$ colonies), or received a long-term low concentration (LongLow; $n = 20$), a medium-term medium concentration (MedMed; $n = 18$) or a short-term high concentration (ShortHigh; $n = 13$) of formic acid. An asterisk indicates a significant treatment x time interaction for each treatment compared to the control ($P \leq 0.01$).

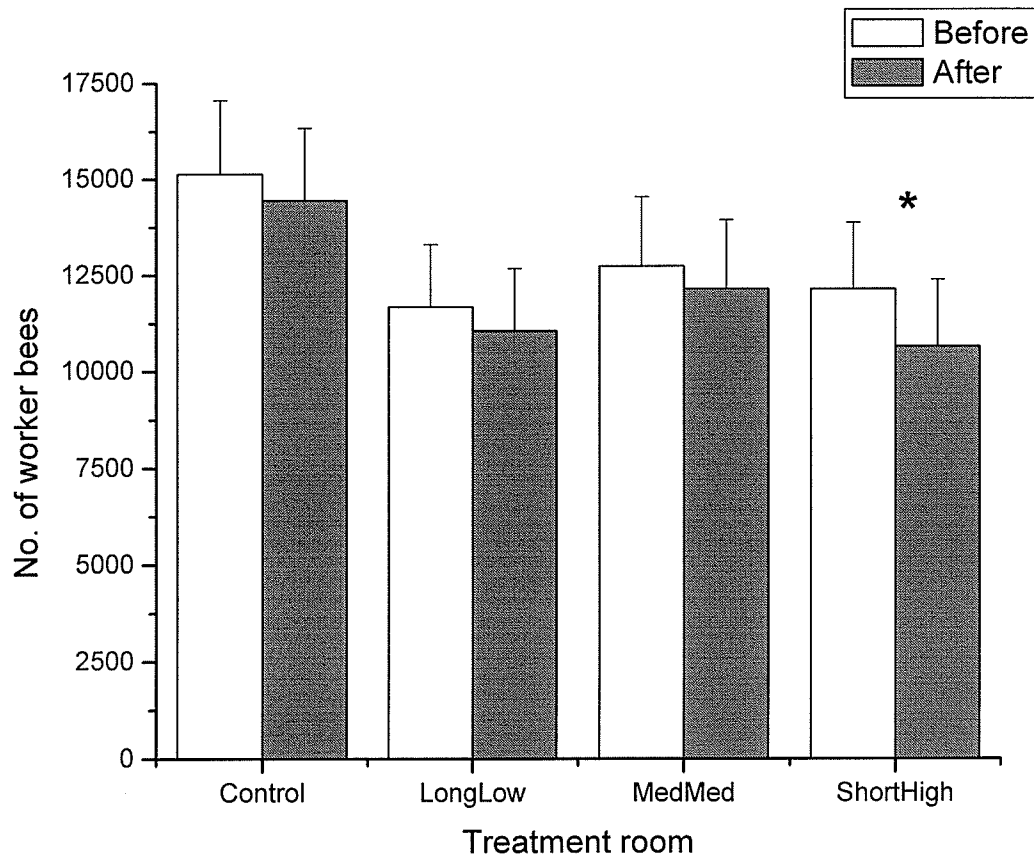


Figure 4.6. Mean honey bee population size (\pm SE) measured before and after wintering in colonies that were untreated (Control; $n = 19$), or received a long-term low concentration (LongLow; $n = 20$), a medium-term medium concentration (MedMed; $n = 18$) or a short-term high concentration (ShortHigh; $n = 13$) of formic acid. An asterisk indicates a significant treatment x time interaction for each treatment compared to the control ($P < 0.05$).

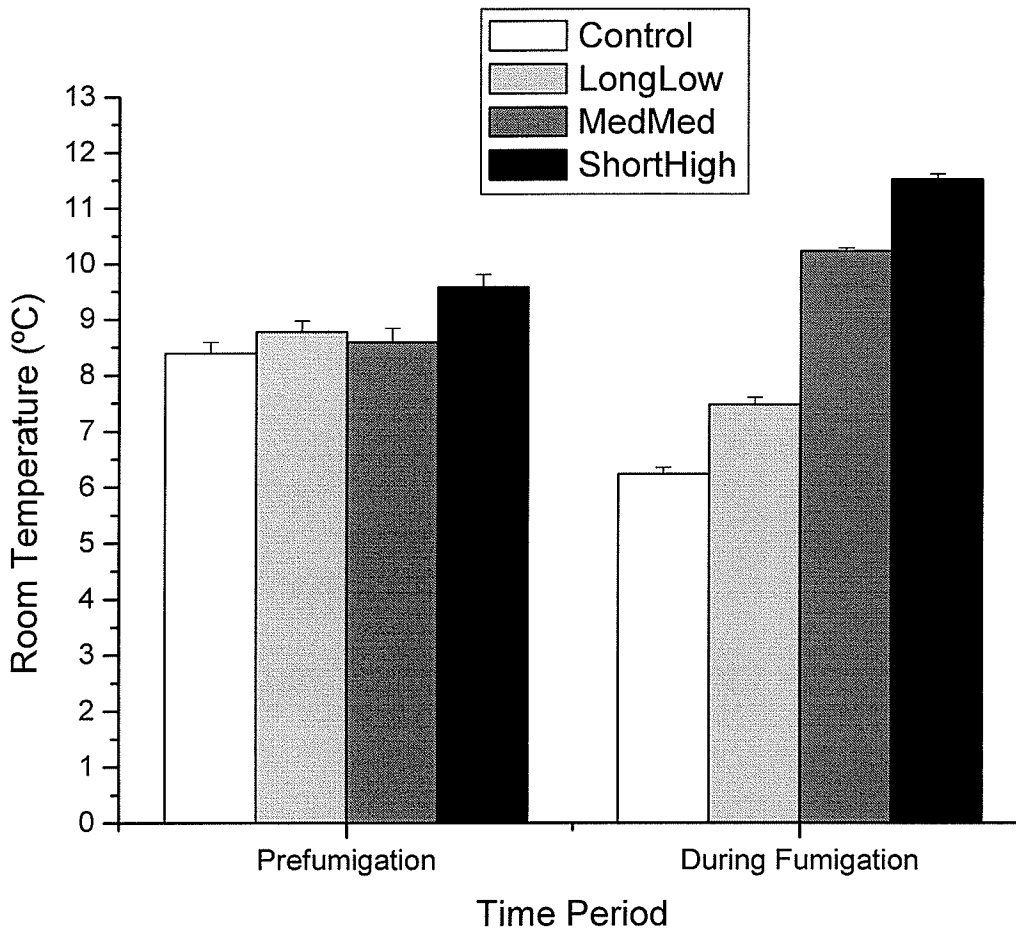


Figure 4.7. Mean temperature (\pm SE) in each experimental treatment room from 2 – 11

January (pre-fumigation) and for the duration of fumigation in experimental rooms containing 21 colonies that were untreated (Control; 27 days), or received a long-term low concentration (LongLow; 27 days), a medium-term medium concentration (MedMed; 10 days) or a short-term high concentration (ShortHigh; 9 days) formic acid. Each bar represents measurements at three locations in each room measured every 30 mins of the measurement period.

CHAPTER 5. LONG-TERM LOW CONCENTRATION FORMIC ACID
FUMIGATION FOR CONTROL OF VARROA MITES, *VARROA DESTRUCTOR*, IN
INDOOR WINTERING HONEY BEE, *APIS MELLIFERA*, COLONIES

ABSTRACT

Long-term low concentration fumigation of honey bee, *Apis mellifera* L., colonies indoors in winter may effectively control the ectoparasitic varroa mite, *Varroa destructor* Anderson and Trueman, without causing queen loss. The objectives of this study were to determine the effect of long-term low concentration formic acid fumigation on 1) varroa mite and queen bee mortality and on room and hive air formic acid concentrations under different ventilation rates and under temporary warming conditions, 2) the cumulative concentration-mortality relationship for formic acid fumigation of varroa mites in honey bee colonies, 3) the effect of hive stacking, honey bee population size and hive weight on in-hive formic acid concentrations and 4) the absorption of formic acid by capped and uncapped honey.

Indoor winter fumigation with a low concentration (7 - 17 ppm) of formic acid in room air for 47 or 65 days significantly increased varroa mite mortality without increasing worker or queen mortality, even during periods of increased room temperature. The cumulative concentration-mortality relationship between varroa mites and formic acid based on in-hive concentrations at room temperatures of 4 °C showed that the CT₅₀ and CT₉₅ for varroa mites were 76 and 728 ppm*days, respectively, but not all colonies reached the CT₉₅ within the fumigation period. Formic acid concentration in room and hive air did not equilibrate even after 65 days. Stacking height, honey bee

population size, and absorption by honey all appeared to contribute to variability in in-hive formic acid concentration.

INTRODUCTION

Formic acid fumigation of honey bee, *Apis mellifera* L., colonies in indoor wintering facilities for control of varroa mites, *Varroa destructor* Anderson and Trueman, is a feasible alternative in northern climates (Underwood and Currie, 2004). This control method is highly desirable because it allows the exploitation of the pause in bee and mite reproduction that occurs in winter (Harris, 1980), reduces labor costs compared to conventional applications, and allows ambient conditions to be controlled. Indoor wintering is common in the northern prairie region (Gruszka, 1998) where this fumigation technique could be readily incorporated into current practices. Both short-term high concentration treatments and long-term low concentration treatments have been shown to effectively reduce the mean abundance of varroa mites to two or fewer mites per 100 bees (Underwood and Currie, 2004, chapter 3), the spring economic threshold for the Canadian prairie region (Gatien and Currie, 1995; Ostermann and Currie, 2004).

Queen loss during indoor fumigation is a problem when a high concentration of formic acid is used and room ventilation is kept to a minimum (Underwood and Currie, 2004, chapters 3, 4). Queen loss during winter fumigation is clearly associated with high in-hive concentrations of formic acid (chapter 3). When the maximum in-hive formic acid concentration does not exceed 18 ppm, no significant queen loss occurs during treatments, but loss of up to 33 % of queens occurs when the maximum in-hive concentration reaches 60 ppm (Underwood and Currie, 2004, chapter 3). Queen loss is associated with relatively high ambient temperatures in hives treated outdoors (Elzen, 2003). In wintering rooms, small increases in ambient temperature may cause much higher in-hive formic acid concentrations. For example, when a maximum room air

concentration of 75 ppm was recorded, a maximum of 18 ppm in hive air was recorded in an experiment where the maximum ambient temperature was 8 °C (chapter 3). In contrast, when a maximum concentration of 69 ppm in room air was recorded, a maximum of 60 ppm in hive air was recorded in an experiment where the maximum ambient temperature was 15 °C (chapter 4). One means for controlling room temperature during short-term high concentration fumigation is through temperature-dependent variable ventilation. Underwood and Currie (chapter 3) showed that, without decreasing treatment efficacy, this could prevent the high concentrations of acid that are often fatal to queens during high concentration fumigation. It is not known whether ventilation can be used during low concentration fumigation to maintain low room temperatures during periods of increased ambient temperature without affecting the efficacy of the treatment.

During indoor fumigation, formic acid is evenly distributed both within the room and in hives at heights of up to 1.5 m, the equivalent of the height of three single-chambered colonies (Underwood and Currie, 2004, chapter 3). However, when hives were stacked higher and were exposed to high concentrations of formic acid for 10 days, hives in the upper two rows sometimes received a greater cumulative concentration than those in the bottom three rows (chapter 3). Although variable ventilation can minimize the adverse effects of stacking at high concentrations, it is not known whether this technique will work at lower concentrations.

Previous studies have reported that during indoor fumigation, the concentration of formic acid in the room air is significantly higher than in the hive air (Underwood and Currie, 2004, chapters 3, 4). Hive and room air did not reach equilibrium within 27 days (chapter 4). In colonies treated outdoors, honey is known to absorb some of the acid

(Stoya et al., 1986; Hansen and Guldberg, 1988; Liu et al., 1993; Bogdanov et al., 2002).

The concentration of formic acid in hive air may remain lower than that in room air, in part, because of absorption of formic acid by honey. The relationship between the concentration of formic acid in hive air and the length of time that formic acid will continue to be absorbed by honey has not been established.

The objectives of this study were to determine the effect of long-term low concentration formic acid fumigation on 1) varroa mite and queen bee mortality and on room and hive air formic acid concentrations under different ventilation rates and under temporary warming conditions, 2) the cumulative concentration-mortality relationship for formic acid fumigation of varroa mites in honey bee colonies, 3) the effect of hive stacking, honey bee population size and hive weight on in-hive formic acid concentrations and 4) the absorption of formic acid by capped and uncapped honey.

MATERIALS AND METHODS

Colony preparation

On 21 November 2002, 80 honey bee colonies contained in single Langstroth hive bodies were assessed for hive weight, honey bee population size, and varroa mite mean abundance and were brought indoors where they were placed randomly in stacks in a large holding room (5.2 x 7.2 x 2.5 m). Initial mean abundance of varroa mites was measured from a sample of 200-300 workers from each colony by an alcohol wash method using a mechanical shaker (Gatien and Currie, 2003). 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 10 January 2003, colonies were randomly assigned to one of four experimental treatment rooms. The hives were placed in four stacks of five hives each with the entrances perpendicular to the air distribution tube (Underwood and Currie, 2004). Each hive had a completely open bottom entrance (37 x 2.5 cm) and a small top entrance (5.7 x 1.8 cm). A white board covering the entire surface of the bottom board was placed in each hive to collect all worker and queen bees and varroa mites falling from the cluster. To collect white board samples within the rooms during fumigation, a low pressure ambient air breathing apparatus (Model 1023-P152G-G608X, Gast Manufacturing Corp., Benton Harbor, MI, USA) with continuous flow supplied air hood (Model R799, Wilson Safety Products, Reading, PA, USA) was used. This allowed the experimenter to enter the experimental rooms safely during fumigation at the relatively low concentrations of formic acid used in this experiment. White board contents from all colonies were collected daily for 80 days from the start of fumigation until the colonies were moved outdoors in the spring.

Fumigation

Fumigation tests were conducted in four 3.0 x 1.7 x 2.5 m treatment rooms in the wintering building at the University of Manitoba in Winnipeg, Manitoba, Canada (N 49° 48' 32", W 97° 07' 37"). Each room simulated the air flow conditions of a standard commercial wintering building with a fan jet distribution system (Gruszka, 1998), but was modified to allow formic acid fumigation (Underwood and Currie, 2004). 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 x 25.0 x 5.5 cm) of liquid containing three T-shaped humidifier plates (11.5

x 16.7 x 0.2 cm, 64-3119-0, Dundas-Jafine Industries Ltd., Weston, ON, Canada) was placed in front of a fan (10 cm diameter window fan, Holmes Products Corp., Milford, MA, USA). A 10-liter plastic bag (Reliance Products Ltd., Winnipeg, MB, Canada) was connected to the pan by a polyethylene tube (4.3 mm inner diameter, Anderson Barrows Metals Corp., Palmdale, CA, USA). 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, ON, Canada). A wall-mounted heater attached to a thermostat maintained each treatment room at approximately 4 °C.

The four experimental treatment rooms consisted of an untreated control room and three rooms fumigated with a low concentration of formic acid. All treatment rooms received a constant minimum ventilation rate of 0.4 L/s/m³. The control room and two of the fumigated rooms had variable ventilation with the minimum ventilation supplemented with an additional fan set on a thermostat that increased the ventilation rate to 8.2 L/s/m³ when room temperature rose above 6 °C. One variable ventilation and one minimum ventilation room were fumigated for a total of 47 days. To simulate the effect of a mid-winter warming spell, the heaters in these two rooms were set to raise the temperature to 10 °C for the last six days of fumigation (Days 42 - 47). The third treated room was fumigated for 65 days with heaters set to a constant 5 °C. A warm-up occurred naturally as outside temperatures increased to daily maxima of up to 13 °C starting on day 55 of fumigation and continuing for the duration of the fumigation period.

For each formic acid treatment room, fumigation began on 18 January 2003 with 6 L of 25% formic acid in the reservoir bag that was allowed to trickle into the pan. Reservoir bags were refilled with 25% formic acid as required throughout the fumigation

period. In total, 13.9 and 16.6 L 25% formic acid were used in the variable ventilation 47-day treatment room and variable ventilation 65-day treatment rooms, respectively. The amount used in the minimum ventilation 47-day treatment room was not measured.

Formic acid concentration measurements

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, USA) calibrated using Dräger tubes (Tube No. 6722101 with Accuro 9022003 gas detector pump, National Dräger, Pittsburgh, PA, USA) or by using Dräger tubes directly. 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 Scientific Ltd., Ottawa, ON, Canada). Measurements were taken from within hives at a point near the center of the honey bee cluster and from the room at a height of 1.5 m from 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. A HOBO[®] data logger (Onset Computer Corp., Bourne, MA, USA) was placed in the center of each treatment room near the opening of the air-sampling tube, where temperature and humidity were measured every 30 min throughout the 80-day examination period.

Because of the large number of hives tested and the length of time required to collect gas samples using the *PortaSens II* sampling device, all hives could not be sampled in a single day. Therefore, one stack (five hives) per fumigated treatment room was sampled each day with each hive being sampled approximately every 6 days. In

addition, all hives were sampled on the same day using Dräger tubes on days 5, 17, 29, 41. An additional Dräger tube sample was taken from all hives in a treatment room just prior to ending fumigation for that treatment (Day 47 or 65). Room air in all acid-treated rooms was sampled daily using the *PortaSens II* sampling device. Formic acid concentration measurements at each site continued after fumigation ceased until values were ≤ 2 ppm.

Final assessment

Colonies were moved outdoors on 9 April 2003 at which time post-treatment measurements of hive weight, honey bee population size, and varroa mite mean abundance were obtained, as described above. To prevent cross-contamination between treated and untreated colonies, untreated colonies were placed in a separate group about 1 km from the treated colonies. To quantify treatment efficacy, one coumaphos strip was placed in each colony on 9 April. A screened bottom board was set in place over a white sticky board to collect all fallen mites during a two-week period. The number of mites collected was 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 mortality to day i by the total number of mites that dropped during the entire trial.

Honey analysis

Forty-eight full honey frames were sampled on 18 January 2004 before being randomly assigned to one of two experimental treatment rooms; the variable ventilation 47-day and 65-day treatment rooms. For analysis, approximately 50 mL of honey and

wax was collected from one side of each numbered frame and placed in a 50 mL polypropylene centrifuge tube with screw top lid (05-539-6, Fisher Scientific Ltd.). Half of the frames were then uncapped using a cappings scratcher. Capped and uncapped frames were randomly assigned to a position within one of the two ventilated treatment rooms. Frames were hung 15 cm apart on stands stacked against the back wall of the treatment room. Three capped and three uncapped frames were removed from each room after 10, 20, 30 or 40 days of fumigation, sampled, and then discarded. Samples were stored in sealed centrifuge tubes at 5 °C until analysis.

The amount of formic acid in honey samples was assessed using the Boehringer Mannheim Enzymatic BioAnalysis Formic acid UV-method (R-Biopharm, Darmstadt, Germany) according to the methods of Liu et al. (1993) with a SPECTRAmax 340PC microplate spectrophotometer (Molecular Devices Corp., Sunnyvale, CA, USA). A 5 g sample of honey was dissolved in deionized distilled water. The pH of each sample was adjusted to between 7 and 8 using sodium hydroxide in deionized distilled water. For analysis, reaction mixtures 2 and 3 were prepared as per the manufacturer's instructions. Each well of the microtiter plate (Becton Dickinson and Company, Lincoln Park, NJ, USA) received 100 μ L "reaction mixture 2" and 190 μ L deionized distilled water. To that, 10 μ L of the honey solution or a standard (serial dilutions from 0.008 to 1 mg/mL) was added. Samples and standards were prepared in the morning and analyzed in the afternoon of the same day. The plate was agitated at room temperature on the plate reader. After 5 min, 5 μ L of "reaction mixture 3" was added rapidly to all wells. The plate was read at 340 nm, agitated every 5 min for 20 min, and then read again. The

difference in absorbance from the initial to the final reading was used to determine the formic acid content.

Statistical analysis

The effects of stacking height on in-hive CT product and the honey bee population were analyzed by analysis of variance (ANOVA) using a randomized block design (PROC GLM; SAS Institute Inc., 1999). When a significant treatment room effect was found, Duncan's multiple range test was used to compare differences between means (SAS Institute Inc., 1999). The correlation between both hive weight and honey bee population size (as measured in spring) and in-hive CT product was analyzed using pooled data from all three acid-fumigated rooms using PROC CORR (SAS Institute Inc., 1999). The effect of treatment room on in-hive formic acid concentration over the 47 days of simultaneous fumigation in all treatment rooms was analyzed using a split-plot ANOVA with treatment room and replicate as the main plot factors and time as the subplot factor (PROC GLM; SAS Institute Inc., 1999).

The effect of treatment room on varroa mite and worker bee mortality over the 80 days of sampling was examined using a split-plot ANOVA (PROC GLM; SAS Institute Inc., 1999). Varroa mite values were weighted by the total number of mites present at the start of fumigation in each colony (described above). Treatment room and replicate were the main plot factors and time was the subplot factor. When significant treatment room x time interactions were found, the interactions within time periods were examined. Treatment room x time interactions were examined within specific time periods of interest (days 1 - 41, 42 - 47, and 65 - 80) by analysis of variance of the average daily mortality rate of varroa mites during that time period weighted by the estimated total

number of mites present at the start of fumigation in each colony. Average daily mortality rate of varroa mites for each colony was obtained by linear regression for each time period (PROC REG; Allen, 1983; SAS Institute Inc., 1999). The effect of treatment room on cumulative varroa mortality as of the last day of the experiment (day 80) was also analyzed by ANOVA. When significant treatment room differences were found ($P < 0.05$), a Student-Neumann-Keuls (SNK) multiple range test was used to compare differences between means (SAS Institute Inc., 1999).

The effect of treatment room on queen survival was analyzed using Fisher's exact test and the effect of treatment room on colony survival was analyzed using the Chi-square test (PROC FREQ; SAS Institute Inc., 1999).

The effect of treatment on mean abundance of varroa mites, the worker bee population size, and hive weight was analyzed as a Before-After Control-Impact (BACI) design (Stewart-Oaten et al., 1986; Smith, 2002) where hives were treated as replicates using a repeated measures ANOVA with an unstructured covariance structure (PROC MIXED; SAS Institute Inc., 1999). Varroa mite mean abundance was analyzed after arcsine transformation.

Similarly, a BACI design was used with a split-plot ANOVA to analyze the change in the concentration of formic acid in honey samples at each sampling date (10, 20, 30 or 40 days). Capping status and replicate were the main plot factors and time (before and after fumigation) was the sub-plot factor (PROC GLM; SAS Institute Inc., 1999).

RESULTS

Formic acid concentration

Formic acid concentration in room air during the first 41 days of fumigation when all treatments were applied simultaneously and without heating, averaged 10 ± 1 ppm (range 1 - 26 ppm) in the variable ventilation 47-day treatment room for a CT product of 520 ppm*days, 10 ± 1 ppm (range 2 - 19 ppm) in the minimum ventilation 47-day treatment room for a CT product of 464 ppm*days, and 17 ± 1 ppm (range 3 - 35 ppm) in the variable ventilation 65-day treatment room for a CT product of 1014 ppm*days. When the thermostat was intentionally increased to 10 °C from days 42 - 47 in the 47-day treatment rooms, the formic acid concentration in room air averaged 7 ± 3 ppm (range 1 - 17 ppm) in the variable ventilation 47-day treatment room and 9 ± 2 ppm (range 3 - 16 ppm) in the minimum ventilation 47-day treatment room and when natural warming increased room temperature during late winter fumigation (days 55 - 65) the formic acid in room air averaged 3 ± 0.4 ppm (range 1 - 6 ppm) in the variable ventilation 65-day treatment room. The formic acid concentration in the room air remained substantially higher than the concentration within the hives throughout the fumigation period, except during the period of natural warming in the variable ventilation 65-day treatment room (Fig. 5.1).

Formic acid concentration in hive air was similar in all acid-fumigated treatment rooms during the first 41 days of fumigation when all treatments were applied simultaneously and without heating ($F = 0.03$, $df = 2, 38$, $P > 0.05$; Table 5.1). The average in-hive CT product was 211 ± 59 ppm*days (range 19 - 782 ppm*days) in the variable ventilation 47-day treatment room, 230 ± 34 ppm*days (range 24 - 623

ppm*days) in the minimum ventilation 47-day treatment room, and 256 ± 60 ppm*days (range 52 – 802 ppm*days) in the variable ventilation 65-day treatment room. In-hive CT product differed with stacking height of the hive ($F = 4.12$, $df = 4$, 53 , $P < 0.05$), but not with treatment room ($F = 1.50$, $df = 2$, 53 , $P > 0.05$). Hives in row 5 accumulated a significantly higher CT product than those in rows 1 - 4 (SNK; Fig. 5.2). Stacking height did not affect honey bee population size ($F = 1.18$, $df = 4$, 53 , $P > 0.05$). In-hive CT product was negatively correlated with spring honey bee population size (-0.38 , $P < 0.01$), but not with final hive weight ($r = -0.01$, $P > 0.05$).

Varroa mite daily mortality

Varroa mite mortality was significantly affected by treatment room over the 80 days of the experiment as indicated by a significant treatment x time interaction ($F = 10.18$, $df = 237$, 2922 , $P < 0.0001$; Fig. 5.3). Cumulative mite mortality on day 80 was significantly affected by treatment room ($F = 10.43$, $df = 3$, 37 , $P < 0.0001$) with all three acid fumigation treatments killing more mites than the control (Fig. 5.3).

The effect of treatment room on the average daily varroa mite mortality rate was assessed within three time periods; days 1 - 41 when treatments took place in the absence of heating, days 42 - 47 when room temperature was intentionally increased in the two 47-day treatment rooms, and days 65 - 80 after all treatments had ceased. During days 1 - 41, when all three formic acid-treated rooms were fumigated simultaneously and without heating, fumigation treatment significantly increased daily varroa mite mortality rate ($F = 4.28$, $df = 3$, 54 , $P < 0.01$; Table 5.1). In all three acid-fumigated rooms, daily varroa mite mortality rate was increased relative to the control (SNK; Table 5.1). During days 42 - 47, the 5 days of increased room temperature in the two 47-day treatment

rooms, varroa mite mortality rate also differed with treatment room ($F = 16.37$, $df = 3$, 35 , $P < 0.0001$; Table 5.1). In the two intentionally heated rooms (both 47-day treatment rooms), varroa mite mortality rate was increased relative to the control, with the mortality rate being greater in the minimum ventilation 47-day treatment room than in the variable ventilation 47-day treatment room. During this time period, mortality in the 65-day treatment room was not different from the control or 47-day variable ventilation treatment rooms, but was significantly less than in the 47-day minimum ventilation treatment room. During days 66 - 80, after fumigation ceased in all treatment rooms, there was no significant difference in varroa mite mortality rate between treatment rooms ($F = 1.38$, $df = 3$, 37 , $P > 0.05$; Table 5.1).

The relationship between cumulative concentration of formic acid and varroa mite mortality in 60 acid-fumigated colonies was used to estimate the CT_{50} and CT_{95} products for fumigation with a low concentration of formic acid within the wintering building (Fig. 5.4). The CT_{50} was 76 ppm*days and the CT_{95} was 728 ppm*days (Fig. 5.4).

Colonies in the control room contained an average of 219 ± 99 varroa mites at the start of fumigation (range 14 – 1133, $n = 14$). Colonies in the variable ventilation 47-day treatment room contained an average of 135 ± 63 varroa mites (range 2 – 962, $n = 15$). Colonies in the minimum ventilation 47-day treatment room contained an average of 137 ± 100 varroa mites (range 3 – 1339, $n = 15$). Colonies in the variable ventilation 65-day treatment room contained an average of 92 ± 30 varroa mites (range 7 – 397, $n = 15$).

Worker bee daily mortality

Fumigation treatment did not affect daily worker bee mortality rate during any time period (Days 1 - 41; $F = 1.26$, $df = 3$, 55 , $P > 0.05$; Days 42 - 47; $F = 1.75$, $df = 3$,

55, $P > 0.05$; Days 66 - 80; $F = 1.39$, $df = 3, 21$, $P > 0.05$) with an average mortality rate of 0.3 ± 0.03 % per day. Colonies in the control room contained an average of $6,973 \pm 554$ worker bees at the start of the fumigation period (range 2,871 – 11,337; $n = 14$) of which 16 ± 3 % dropped during the 80 days of sampling. Colonies in the variable ventilation 47-day treatment room contained an average of $8,076 \pm 1,198$ bees (range 1,334 – 16,528; $n = 15$) of which 16 ± 3 % dropped. Colonies in the minimum ventilation 47-day treatment room contained an average of $6,174 \pm 1,214$ bees (range 1,064 – 18,269; $n = 15$) of which 28 ± 6 % dropped. Colonies in the variable ventilation 65-day treatment room averaged $7,387 \pm 1,108$ bees (range 1,012 – 15,722; $n = 15$) of which 22 ± 6 % dropped.

Queen bee and colony mortality

Queen drop was not affected by formic acid fumigation ($P > 0.05$). One queen dropped in the variable ventilation 65-day treatment room and none were lost in any other treatment room. Colony survival also was not significantly affected by formic acid fumigation ($P > 0.05$) with 14 of 20 colonies surviving in the control and 15 of 20 colonies surviving in each of the formic acid-fumigated treatment rooms.

Varroa mite mean abundance

The change in mean abundance of varroa mites over the course of the experiment was not affected by formic acid fumigation as indicated by a non-significant treatment room x time interaction ($F = 0.04$, $df = 3, 110$, $P > 0.05$; Table 5.2). However, in all three acid-fumigated rooms, the mean abundance of varroa mites was below 2 mites per 100 bees in the spring.

Worker bee population size

Worker bee population size was not affected by formic acid fumigation treatment as indicated by a non-significant treatment x time interaction ($F = 0.02$, $df = 3, 110$, $P > 0.05$; Table 5.2).

Hive weight

Hive weight change over the course of the experiment was not affected by treatment room as indicated by a non-significant treatment x time interaction ($F = 0.73$, $df = 3, 110$, $P > 0.05$; Table 5.2).

Room temperature and humidity

Room temperature and humidity during each time period of interest can be found in table 5.1. During the first 41 days of the trial, when all three formic acid-fumigated rooms were fumigated simultaneously and without heating, temperature was similar in all treatment rooms, while humidity was higher in acid-fumigated rooms than in the control room. Intentional heating of the 47-day treatment rooms from day 42 - 47 increased room temperature and decreased humidity relative to the control and 65-day treatment rooms, which were not heated. After fumigation ceased (Days 66 - 80), all treatment rooms had similar temperatures and humidities.

Formic acid concentration in honey

The presence or absence of wax cappings did not affect formic acid absorption by honey at any sampling date (10 days; $F = 1.22$, $df = 1, 10$, $P > 0.05$; 20 days; $F = 2.28$, $df = 1, 10$, $P > 0.05$; 30 days; $F = 2.12$, $df = 1, 10$, $P > 0.05$; 40 days; $F = 0.13$, $df = 1, 10$, $P > 0.05$; Fig. 5.5). Formic acid concentration in honey was significantly greater than the baseline level in frames fumigated for 20, 30 and 40 days (20 days; $F = 27.30$, $df = 1, 10$,

$P < 0.001$; 30 days; $F = 28.89$, $df = 1, 10$, $P < 0.001$; 40 days; $F = 34.44$, $df = 1, 10$, $P < 0.001$), but not in frames fumigated for 10 days ($F = 2.89$, $df = 1, 10$, $P > 0.05$; Fig. 5.5).

DISCUSSION

Indoor winter fumigation with a low concentration of formic acid for 47 or 65 days significantly increased varroa mite mortality without increasing worker or queen mortality, even during periods of heating. The cumulative concentration-mortality relationship between varroa mites and formic acid based on in-hive concentrations at room temperatures of 4 °C showed that the CT_{50} and CT_{95} for varroa mites were 76 and 728 ppm*days, respectively, but not all colonies reached the CT_{95} . Formic acid concentration in room and hive air did not equilibrate even after 65 days. Stacking height, honey bee population size, and absorption by honey all appeared to contribute to variability in in-hive formic acid concentration.

The cumulative concentration-mortality relationship between formic acid and varroa mites under a low concentration of formic acid as used in this study was comparable to those established for high concentrations of formic acid (chapter 3). Both studies showed that varroa mite mortality is quite marked as the in-hive CT product approached 110 ppm*days. The curve established from this study becomes noticeably less steep at higher CT products. Under fumigation with a low concentration of formic acid, the CT_{50} and CT_{95} products (76 and 728 ppm*days, respectively) were higher than those established under high concentrations (49 and 111 ppm*days, respectively) (chapter 3). Underwood and Currie (2003) showed that “at most temperatures the greatest fumigation efficiency occurred at lower concentrations of formic acid.” Our

results suggest that when fumigating at temperatures of 4 °C, using low concentrations of formic acid might be less efficient than using high concentrations, but with low concentration fumigation, over 95% of varroa mites could be killed if the necessary CT product was consistently realized in hive air.

The concentration-mortality relationship within each hive was highly variable (Fig. 5.4) which was due, in part, to the low number of varroa mites in some colonies. For example, one colony where 100 % varroa mite mortality occurred at a final CT product of 189 ppm*days contained only 2 mites and another colony where only 50 % mite mortality occurred at a final CT product of 550 ppm*days contained only 27 varroa mites. Thus, unweighted proportions based on these small sample sizes may account for the large divergence in some colonies from the line which is weighted. In addition, because the total number of mites present at the start of fumigation in each colony was determined using post-treatment mite drop, mites that were killed during formic acid fumigation, but dropped onto the white board after fumigation when the bees cleaned the hive in spring, would have decreased the efficacy values. This effect would have had a larger influence on colonies with small mite populations than on those with large populations.

Different combinations of formic acid treatment and ventilation level affected varroa mite mortality. During intentional heating, mite mortality in the minimum ventilation 47-day treatment room was greater than in the heated variable ventilation 47-day treatment room and unheated variable ventilation rooms. We cannot attribute this to a higher in-hive formic acid concentration, as there was no effect of treatment on in-hive concentration. However, because concentration measurements were taken once daily and

ventilation was not monitored, we do not know whether the measurements were taken during times of increased or minimum ventilation. Further work is needed to determine whether differences in-hive temperature or in-hive formic acid concentration or air flow rates had a direct effect on varroa mite mortality.

In this experiment, the concentration of formic acid in room air was higher than that measured in hive air as is common during indoor winter fumigation (Underwood and Currie, 2004, chapters 3, 4). Surprisingly, room and hive air did not reach equilibrium even after 65 days of fumigation when a low concentration of formic acid was used. In the ventilated 65-day treatment room, the difference in formic acid concentration between room and hive air was smaller in the latter part of the fumigation period, when concentrations of formic acid in room air were substantially lower. After day 55 of fumigation, room temperature increased due to rising temperatures outside causing ventilation fans to turn on (at 6 °C) to keep the room cool. This increased ventilation likely caused the lowered concentration of formic acid in the room air, allowing the room and hive air to approach equilibrium.

The absorption of formic acid by honey, as seen in this experiment, is likely one factor affecting the in-hive concentration of formic acid. Although uncapped honey tended to absorb formic acid more rapidly than capped honey, there was no significant difference in the rate of absorption. Our results show that both capped and uncapped honey absorb formic acid and do not become saturated until more than 10 days of fumigation have passed. During this time, the concentration of formic acid in hive air could be kept lower than the room air due, in part, to this absorption. Similar effects are observed when acid fumigants are utilized in stored grain systems (Sholberg and Gaunce,

1996). However, the formic acid concentration in honey in this study remained relatively stable from day 20 to 40 of fumigation. Because in-hive concentrations did not rise once the honey became saturated, we assume that the bees and/or other hive components continue to absorb and/or adsorb formic acid, thereby lowering the concentration in hive air. Wissen and Maul (1981) found that both bees and wooden hive components have increased formic acid contents as a result of fumigation. Thus, absorption by bees and wooden hive components may be as important a factor as absorption by honey. This is supported by the results of Underwood and Currie (chapter 3) who found that in-hive CT product was negatively correlated with honey bee population size.

Fumigation with low concentrations of formic acid for more than two months did not harm worker or queen honey bees. This finding parallels reports by Underwood and Currie (2004, chapter 4) that showed that long-term low concentration fumigation was not harmful to workers or queens at exposure times of less than 27 days. This study showed that an in-hive CT product of as much as 730 ppm*days given over 65 days did not increase queen mortality. This supports the hypothesis that queen honey bees can withstand exposure to a large cumulative concentration of formic acid if a low concentration is used to prevent the peak formic acid concentration from reaching a lethal limit (chapters 3, 4).

It has been suggested that queen loss may be related to the interaction between formic acid concentration and room temperature during indoor fumigation (chapters 3, 4). During this experiment, room temperature was increased to an average of 8 °C in the last 6 days of fumigation in the 47-day treatment rooms (range 6 - 8 °C) and in the last 10 days in the 65-day treatment room (range 7 - 13 °C). Despite this warming, the rate of

queen loss did not increase, whether the room was ventilated or unventilated. In previous studies, queen loss was seen in combination with formic acid at high concentrations when room temperatures averaged 4 °C (range 1 - 11 °C), 11 °C (range 8 - 18 °C), and 12 °C (range 6 - 15 °C) (chapters 3, 4). It is possible that the increased room temperature is only detrimental to queens if a high concentration of formic acid occurs within the hive.

Our low concentration fumigation utilized 25% formic acid and, thus, may prove to be a safer alternative to using 65 or 85% formic acid. Fumigating indoors in winter is advantageous in that it allows exploitation of the pause in bee and mite reproduction and the subsequent vulnerability of varroa mites on adult bees. Indoor fumigation also allows for considerable control of ambient temperature; a great advantage over the use of formic acid outdoors. While outdoor use of formic acid in fall has been somewhat useful as a varroa mite control method (Calderone, 1999; Calderone and Nasr 1999) it is not always reliable (Gatien and Currie, 2003; Currie and Gatien, in prep.) and does not provide protection against late season robbing of highly infested colonies. Winter treatments would reduce the need for spring applications that are required when fall treatments fail.

Long-term low concentration fumigation with formic acid in indoor wintering buildings is safe for worker and queen bees while being an effective varroa mite control method. Allowing room temperature to be controlled with ventilation decreased the efficacy somewhat, but this deficiency was overcome with a longer fumigation period and a slightly higher formic acid concentration in room air. For 95 % control using a low concentration, a CT product of 728 ppm*days was required, although most hives did not reach this level within the 47 or 65 days of fumigation. Room and hive air formic acid concentrations did not equilibrate and the variation among hives in a treatment room was

attributed to stacking height, the size of the honey bee population, and absorption by honey. Reduction in in-hive variability would allow more efficient and efficacious treatments. However, this treatment allowed honey bee colonies to start the spring with a mean abundance of varroa mites of 1 mite per 100 bees, which is below the spring economic threshold for the Canadian prairies.

Table 5.1. Average (mean \pm SE) daily varroa mite mortality rate, formic acid concentration in hives, temperature, and relative humidity in rooms that were untreated with variable ventilation (control), treated with formic acid for 47 days with variable ventilation (varvent47), treated for 47 days with minimum ventilation (minvent47), or treated for 65 days with variable ventilation (varvent65). Numbers in parentheses indicate the maximum-recorded value for that measure. Average daily varroa mite mortality rate was determined by linear regression for each colony within each treatment room during each time period and weighted by the total number of varroa mites present at the start of fumigation. For mortality rate, means followed by the same letter within a time period are not significantly different from each other (SNK; $P > 0.05$).

Time Period	Control	Varvent47	Minvent47	Varvent65
Mortality Rate (%/day)				
Days 1 - 41	0.3 \pm 0.03 a	1.0 \pm 0.15 b	1.2 \pm 0.12 b	1.0 \pm 0.17 b
Days 42 - 47 *	0.2 \pm 0.03 a	1.7 \pm 0.23 b	2.8 \pm 0.45 c	1.0 \pm 0.35 ab
Days 65 - 80	0.2 \pm 0.07 a	0.1 \pm 0.05 a	0.2 \pm 0.19 a	0.2 \pm 0.09 a
FA Conc. In-hive (ppm)				
Days 1 - 41	-	4 \pm 0.2 (27)	4 \pm 0.1 (18)	4 \pm 0.2 (24)
Days 42 - 47 *	-	6 \pm 0.7 (28)	7 \pm 0.5 (21)	6 \pm 0.6 (21)
Days 65 - 80	-	-	-	-
Temperature (°C)				
Days 1 - 41	3.5 \pm 0.02	4.1 \pm 0.02	3.5 \pm 0.10 (8)	3.7 \pm 0.02
Days 42 - 47 *	3.0 \pm 0.03	7.2 \pm 0.08	6.7 \pm 0.07 (8)	3.7 \pm 0.04
Days 65 - 80	5.6 \pm 0.07	5.8 \pm 0.07	5.3 \pm 0.07	5.8 \pm 0.08
Humidity (%RH)				
Days 1 - 41	27 \pm 0.1	41 \pm 0.1 (57)	42 \pm 0.1 (56)	47 \pm 0.1 (59)
Days 42 - 47 *	26 \pm 0.1	34 \pm 0.2 (41)	36 \pm 0.2 (43)	46 \pm 0.2 (54)
Days 65 - 80	40 \pm 0.1	41 \pm 0.3 (68)	44 \pm 0.3 (71)	41 \pm 0.3 (69)

* Period in which the Varvent47 and Minvent47 treatment rooms were intentionally heated.

Table 5.2. Mean (\pm SE) honey bee population size, hive weight, and varroa mite mean abundance in colonies that were untreated (control) or treated with a low concentration of formic acid with variable ventilation for 47 days (varvent47), with minimum ventilation for 47 days (minvent47), or with variable ventilation for 65 days (varvent65). Colonies surviving until spring were measured before and after wintering; $n = 14$ colonies in the control; $n = 15$ colonies in each acid-fumigated treatment. No significant differences were found between treatments ($P > 0.05$).

Treatment	Population size (no. bees)		Hive weight (kg)		Varroa mite mean abundance (no. mites/ 100 bees)	
	Before	After	Before	After	Before	After
Control	10328 \pm 925	5945 \pm 545	31.6 \pm 0.8	24.1 \pm 0.9	1.8 \pm 0.8	2.1 \pm 0.8
Varvent47	11178 \pm 770	7169 \pm 1219	32.1 \pm 0.8	23.6 \pm 0.8	1.2 \pm 0.7	1.3 \pm 0.4
Minvent47	9558 \pm 933	5266 \pm 1226	30.5 \pm 0.9	24.3 \pm 0.8	1.0 \pm 0.7	1.0 \pm 0.5
Varvent65	10854 \pm 1044	6481 \pm 1168	31.9 \pm 0.6	24.2 \pm 0.8	0.9 \pm 0.2	0.9 \pm 0.3

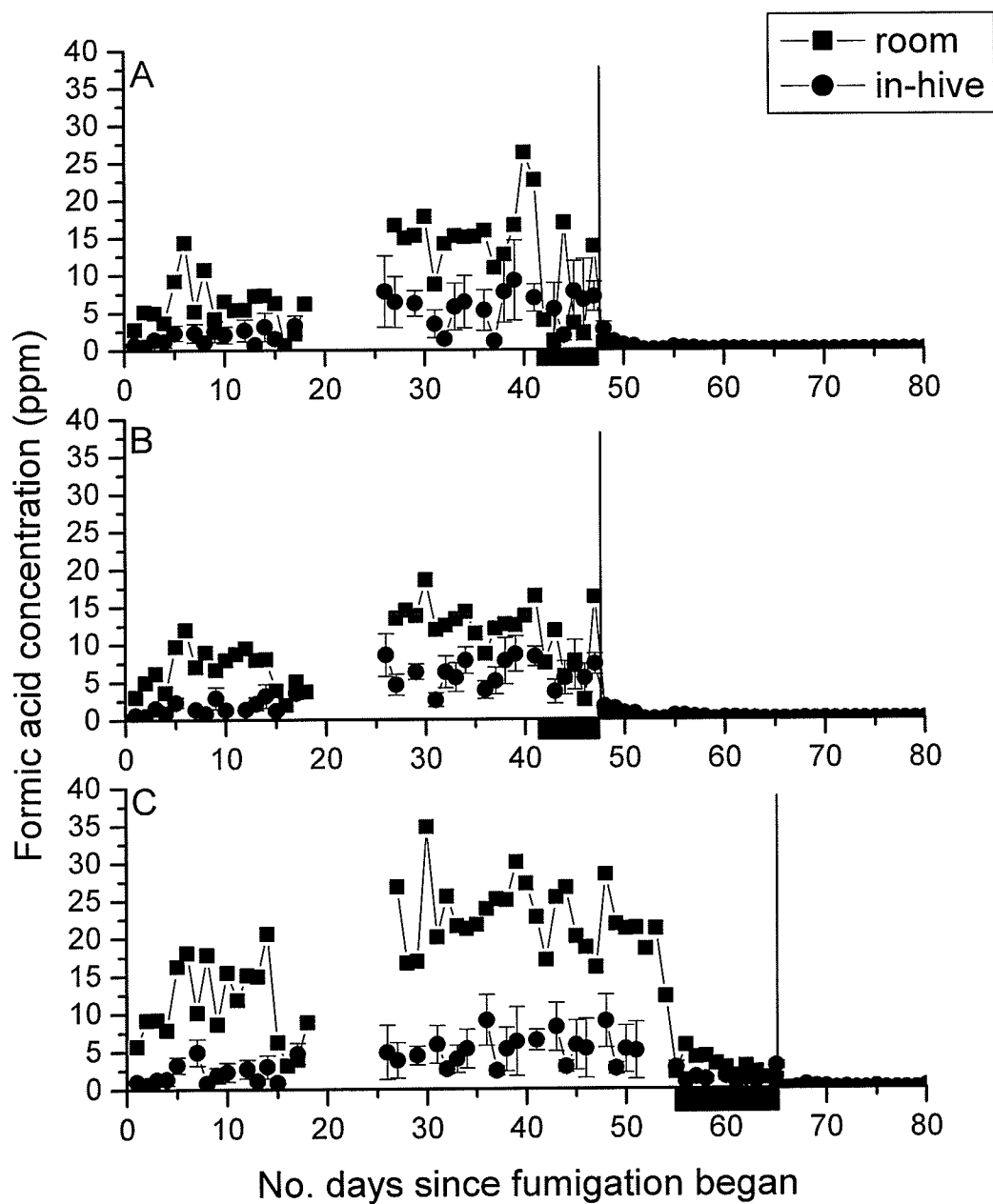


Figure 5.1. Mean (\pm SE) formic acid concentration in room and hive air in the variable ventilation 47-day treatment room (A), minimum ventilation 47-day treatment room (B), and variable ventilation 65-day treatment room (C). Hive air measurements represent a mean of 20 hives in a single treatment room, while

room air measurements represent only one sampling site per treatment room.

Vertical lines indicate the end of fumigation for each treatment room. Horizontal bars indicate the period of heating in each treatment room. No significant differences between treatments were found for either room or hive air ($P > 0.05$).

Data for days 19 – 24 are missing due to technical difficulties.

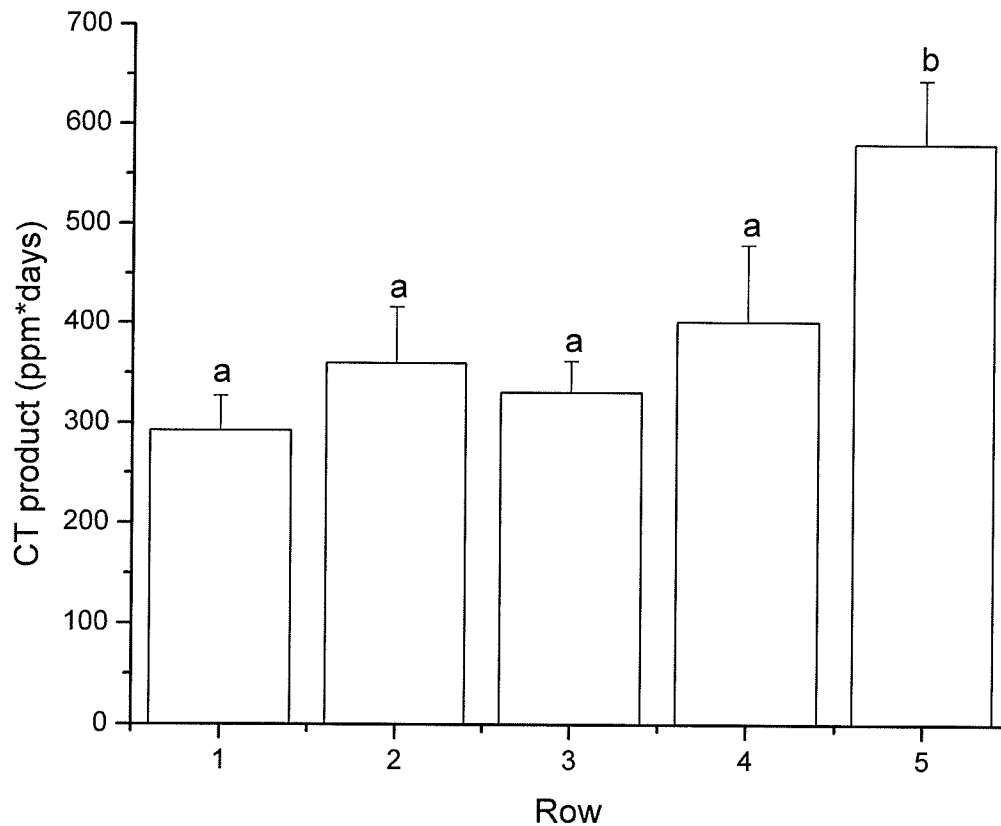


Figure 5.2. Mean in-hive CT product of formic acid (\pm SE) in hives stacked in all three acid-fumigated rooms from the floor (row 1) to the top of the stack (row 5) during the entire fumigation period (47 or 65 days). For each row, $n = 15$ hives. Bars with the same letter are not significantly different from each other (Duncan's; $P > 0.05$).

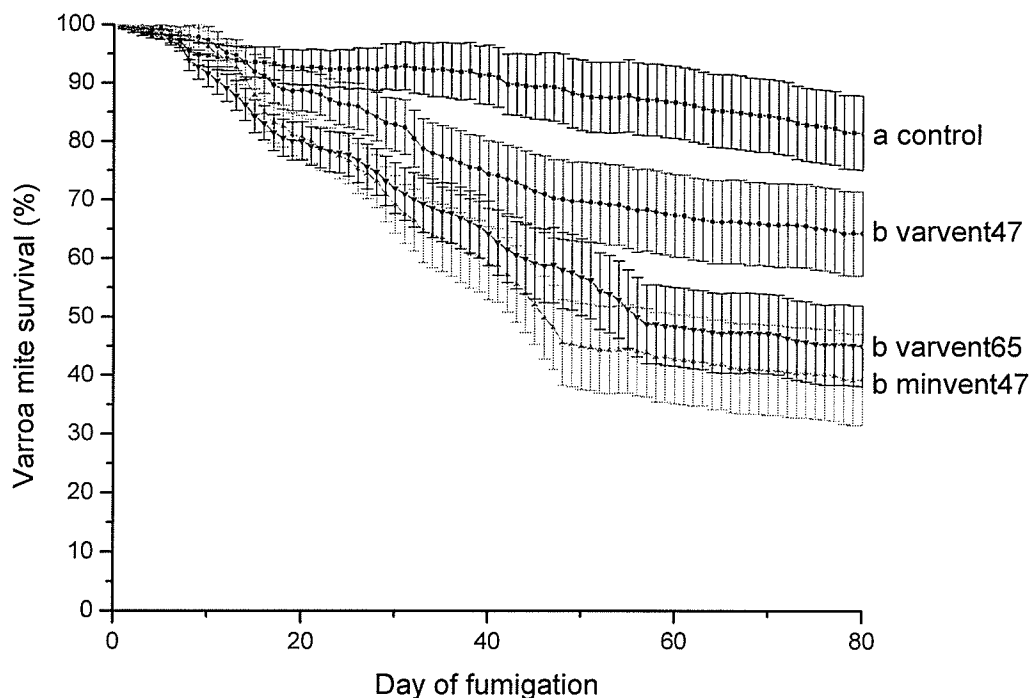


Figure 5.3. Varroa mite survival (weighted lsmean \pm SE) over the course of the experiment in colonies in rooms that were untreated with variable ventilation (control; $n = 14$ colonies or 3059 mites), treated with formic acid for 47 days with variable ventilation (varvent-47; $n = 15$ colonies or 2027 mites), treated for 47 days with minimum ventilation (minvent-47; $n = 15$ colonies or 1788 mites), or treated for 65 days with variable ventilation (varvent-65; $n = 15$ colonies or 1383 mites). For analysis, means are weighted by the estimated total number of varroa mites present at the start of fumigation. Treatments followed by the same letter did not have significantly different varroa mite survival on the last day of the 80-day evaluation period (SNK; $P > 0.05$).

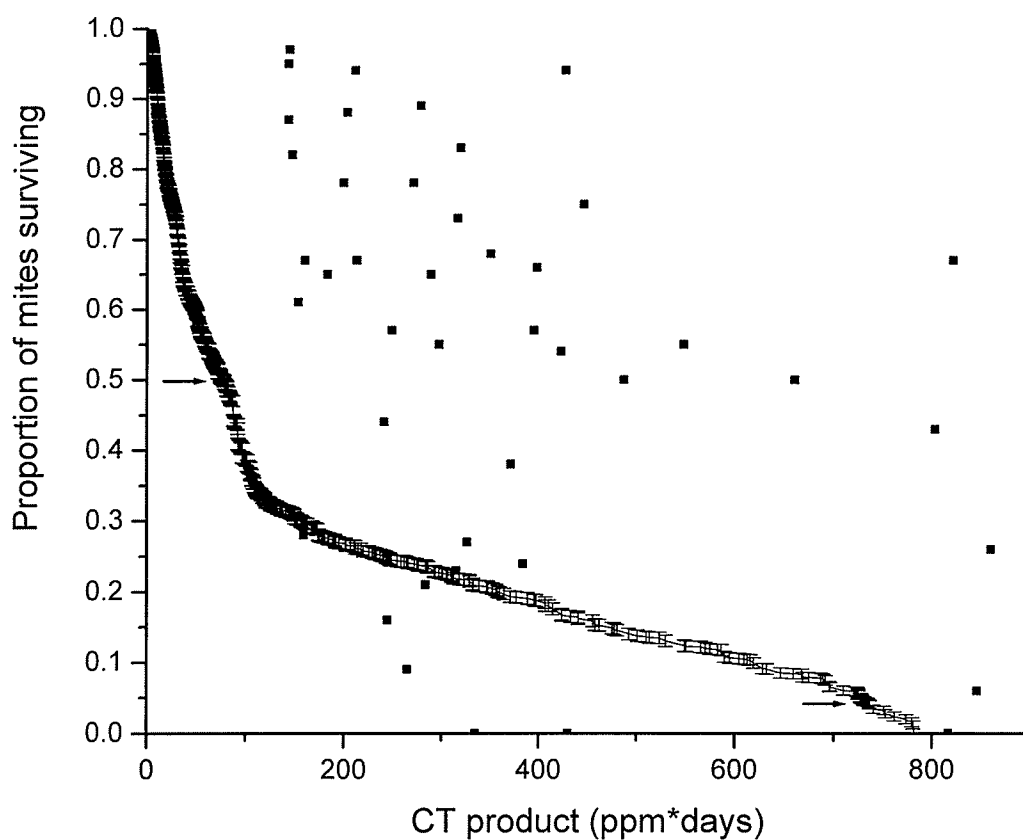


Figure 5.4. Concentration-response curve for varroa mites in colonies treated with a long-term low concentration of formic acid for 47 or 65 days. The line represents the weighted mean (\pm SE) proportion of varroa mites surviving ($n = 5,198$ mites) as each CT product was achieved. The filled squares (■) represent the unweighted total proportion of varroa mites surviving in each hive ($n = 1$ hive for each point) at the final CT product for that hive. The horizontal arrows indicate the point on the line where the CT₅₀ and CT₉₅ are reached.

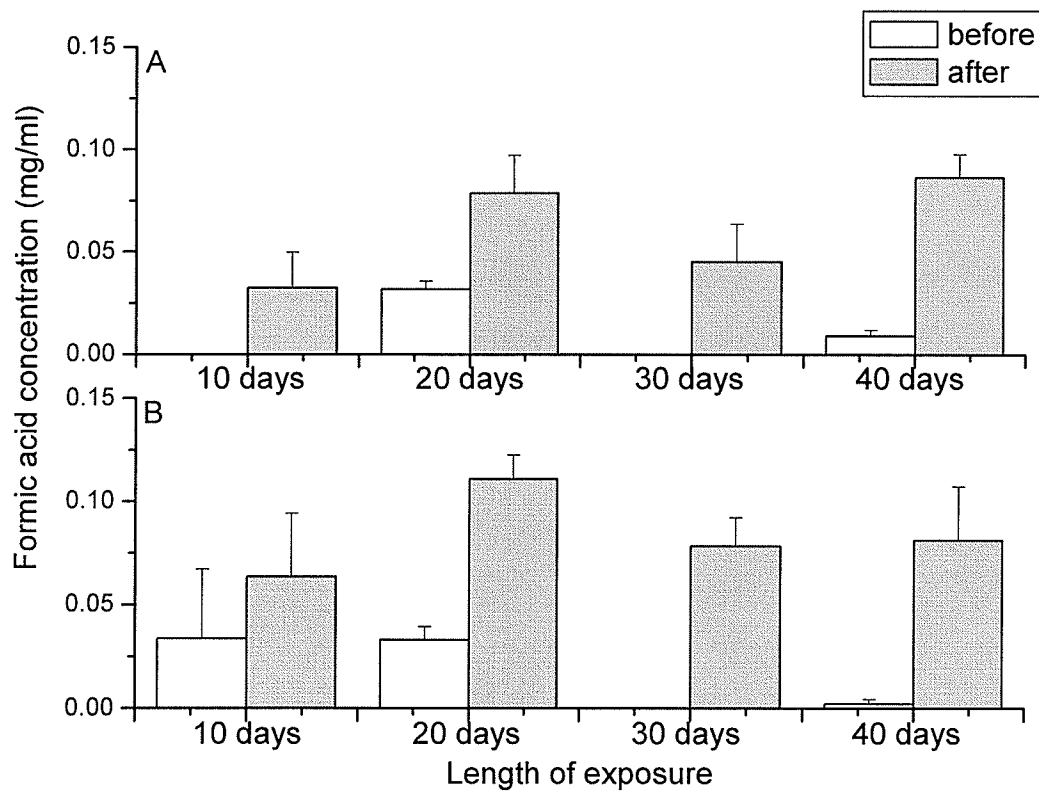


Figure 5.5. Formic acid concentration in capped (A) and uncapped (B) honey before and after exposure to 10 – 17 ppm formic acid in room air for 10, 20, 30 or 40 days. $n = 3$ frames per bar.

CHAPTER 6. INDOOR WINTER FUMIGATION WITH FORMIC ACID FOR
CONTROL OF THE HONEY BEE TRACHEAL MITE, *ACARAPIS WOODI*
(RENNIE), AND NOSEMA DISEASE, *NOSEMA APIS* ZANDER.

ABSTRACT

Indoor fumigation of honey bees, *Apis mellifera* L., with formic acid to control varroa mites, *Varroa destructor* Anderson and Trueman, allows simultaneous fumigation of colonies with little labor input and good efficacy. However, the efficacy of this treatment against tracheal mites, *Acarapis woodi* (Rennie), and nosema disease, *Nosema apis* Zander, needs further study. The objectives of this study are 1) to determine the efficacy of formic acid fumigation for tracheal mite control using both the thoracic slice and live dissection methods and 2) to determine whether indoor fumigation can reliably prevent the build-up of nosema disease in overwintering honey bee colonies. Three experiments were designed to test the efficacy of formic acid as a treatment for tracheal mites and nosema disease indoors in winter.

Indoor winter fumigation of honey bee colonies with formic acid was effective in killing a high percentage of tracheal mites, but did not significantly reduce the proportion of bees infested with tracheal mites over the duration of the experiments. However, under conditions of relatively low or decreasing levels of nosema, fumigation did not significantly suppress the mean abundance of nosema spores relative to the controls. In three separate fumigation experiments, there was no statistical difference between the build-up or maintenance of nosema spore mean abundance over the winter in bees from formic acid fumigated colonies as compared to untreated controls.

INTRODUCTION

Indoor fumigation of honey bees, *Apis mellifera* L., with formic acid to control varroa mites, *Varroa destructor* Anderson and Trueman (Underwood and Currie, 2004, chapters 3, 4, 5) allows simultaneous fumigation of large numbers of colonies with little labor input and good efficacy. Short-term high concentrations and long-term low concentrations have been tested and their effects on worker and queen bees and on varroa mites have been assessed (Underwood and Currie, 2004, chapters 3, 4, 5). However, the efficacy of this treatment against tracheal mites, *Acarapis woodi* (Rennie), and nosema disease, *Nosema apis* Zander, needs further study (Underwood and Currie, 2004).

Formic acid has been successfully used outdoors for the control of tracheal mites (Sharma et al., 1983; Hoppe et al., 1989; Liu, 1991; Wilson et al., 1993; Nelson et al., 1994) where tracheal mite prevalence, or the proportion of bees infested, is significantly reduced using formic acid treatments. However, indoor fumigation does not significantly decrease tracheal mite prevalence (Underwood and Currie, 2004). Underwood and Currie (2004) attributed the apparent lack of tracheal mite reduction to the short duration of fumigation and/or to a lack of sensitivity of the thoracic slice method in quantifying mite mortality.

Tracheal mite mortality can be directly assessed through live dissection of bees where live and dead mites can be separated (Hoppe et al., 1989; Klymochko, 2001). This method of assessment is especially useful for fumigation experiments conducted during the winter in temperate climates. At this time of year, brood production is drastically reduced and there is a subsequent lack of turnover in adult bees (Harris, 1980; Nelson, 1995). Therefore, most of the bees that are exposed to formic acid during winter fumigation are still present when the colonies are tested in

the spring. Tracheal mites that are killed, but not expelled from the bees' bodies, would yield positive results in a thoracic slice, causing an inaccurate estimate of treatment efficacy. Therefore, further studies are required that use live dissections to determine the efficacy of indoor winter fumigation with formic acid for tracheal mite control.

Acid fumigation with a variety of products has shown mixed results for the suppression of nosema disease. DeRuijter and van der Steen (1989) found that nosema spores on glass plates placed in acetic acid-fumigated hives were inviable and unable to infect healthy bees. However, Fries (1990) fumigated spores with formic acid on wax combs and found that they could still cause infection even after four formic acid applications. Ostermann (2002) found that formic acid had no effect on nosema disease in outdoor spring applications, while Underwood and Currie (2004) found that indoor winter fumigation significantly suppressed the build-up of nosema spores over the course of the winter. It is not known whether indoor fumigation can reliably control nosema disease in overwintering honey bee colonies.

Three experiments were designed to test the efficacy of formic acid as a treatment for tracheal mites and nosema disease indoors in winter. Experiment 1 was conducted to test whether room ventilation settings affect tracheal mite survival and nosema disease in full-size colonies fumigated with a high concentration of formic acid. Experiment 2 was conducted to assess three concentrations of formic acid with the same CT product (cumulative concentration) to determine the efficacy of each in controlling tracheal mites and nosema disease. Experiment 3 was conducted to determine whether a long-term low concentration of formic acid affects tracheal mite survival and nosema disease.

MATERIALS AND METHODS

Experimental treatment rooms and general fumigation methods

Fumigation tests were conducted in four 3.0 x 1.7 x 2.5 m treatment rooms in the wintering building at the University of Manitoba in Winnipeg, Manitoba, Canada (N 49° 48' 32", W 97° 07' 37"). Each room simulated the air flow conditions of a standard commercial wintering building with a fan jet air distribution system (Gruszka, 1998), but was modified to allow formic acid fumigation (Underwood and Currie, 2004). 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 x 25.0 x 5.5 cm) of liquid containing three T-shaped humidifier plates (11.5 x 16.7 x 0.2 cm, 64-3119-0, Dundas-Jafine Industries Ltd., Weston, ON, Canada) was placed in front of a fan (10 cm diameter window fan, Holmes Products Corp., Milford, MA, USA). A 10-liter plastic bag (Reliance Products Ltd., Winnipeg, MB, Canada) was connected to the pan by a polyethylene tube (4.3 mm inner diameter, Anderson Barrows Metals Corp., Palmdale, CA, USA). 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, ON, Canada). In all experiments, all rooms received a constant minimum ventilation rate of 0.4 L/s/m³. A wall-mounted heater attached to a thermostat maintained each treatment room at a minimum of 2 °C.

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) calibrated using Dräger tubes (Tube No. 6722101 with Accuro 9022003 gas detector pump, National Dräger, Pittsburgh, PA) or by using Dräger tubes directly. 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 Scientific Limited, Ottawa, Ontario, Canada).

Pre- and post-treatment sampling

For each experiment, pre- and post-treatment samples were collected before the colonies were moved indoors and on the day they were moved outdoors to estimate nosema spore mean abundance and tracheal mite prevalence. Nosema spore mean abundance was assessed according to the methods of Cantwell (1970). Tracheal mite prevalence was assessed by the thoracic slice method according to Delfinado-Baker (1984).

Experiment 1

Eighty-four single-chamber Langstroth hives were obtained from nine commercial beekeepers during the fall of 2001. On 11 December 2001, colonies were brought indoors where they were positioned in stacks placed randomly in a holding room (5.2 x 7.2 x 2.5 m). Each hive had a completely open bottom entrance (37 x 2.5 cm), but lid type varied with beekeeper. On 27 February 2002, the colonies were randomly assigned to four experimental treatment rooms. In each treatment room, the hives were arranged 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.

Experimental treatments consisted of two fumigation levels; fumigated and unfumigated. Within each level of fumigation, pairs were created based on ventilation level. One pair had only the minimum ventilation (0.4 L/s/m^3) while the other pair had variable ventilation with the minimum ventilation supplemented with an additional fan set on a thermostat that increased the ventilation rate to 8.2 L/s/m^3

when room temperature rose above 6 °C. Fumigation began with 1 L 85 % formic acid in the pan and 5 L 85 % formic acid in the reservoir bag. Fumigation began on 4 March and the acid was removed from the air mixing chamber on 11 March 2001, after 7 days of fumigation.

Colonies in the variable ventilation unfumigated room, variable ventilation fumigated treatment room, minimum ventilation unfumigated room ($n = 2$), and minimum ventilation fumigated treatment room that were found to be positive for tracheal mite infestation in the pre-treatment samples were selected for the experiment. A sample of 30 bees was removed from the entrance of each infested colony using a vacuum aspirator on 18 February. Bees were caged with access to 50 % sucrose syrup and kept at room temperature (20 °C) until dissection. Dissections to assess tracheal mite survival were carried out according to the methods of Klymochko (2001). First, the bee's head and prothorax were removed. The bee was then pinned to a wax plate through the metathorax for stabilization while the two mesothoracic tracheal trunks were removed. The tracheae were placed in a drop of distilled water for observation under a binocular microscope at 40X power. All tracheal mites were removed from the tracheae by ripping the tube with a fine wire. Movement indicated that the mite was alive (Hoppe et al., 1989). All mites, dead and alive, were counted in each trachea. Dissections were repeated on 12 March 2002, after formic acid treatments were complete.

Experiment 2

Eighty-four single-chamber Langstroth hives were obtained from a commercial beekeeper (AF; $n = 32$ hives) or the University of Manitoba (UM; $n = 52$ hives) in the late fall of 2001. On 11 December, colonies were weighed and brought indoors where they were positioned in stacks placed randomly in a large holding room

(5.2 x 7.2 x 2.5 m) prior to the start of the experiment. Each hive had a completely open bottom entrance (37 x 2.5 cm) and a small top entrance (5.7 x 1.8 cm). On 28 December 2001, the colonies were randomly assigned to four experimental treatment rooms. Colonies were sorted by beekeeper and initial mean abundance of varroa mites, as measured by an alcohol wash (Gatien and Currie, 2003) and divided into 21 blocks of four hives each. One hive from each block was randomly assigned to a position in each treatment room in one of five adjacent columns against one wall. The middle column contained a stack of five hives and the others contained stacks of four hives.

During the experiment, one room, the control, remained untreated, with no formic acid exposure. The other three rooms were fumigated with formic acid using different treatment regimes. Starting on 11 January, treated colonies were exposed to a low, medium, or high concentration of formic acid until a concentration x time (CT) product of approximately 390 ppm*days (based on room air) was realized in each experimental room. 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 390 ppm*days. This short-term high-concentration treatment consisted of a room air concentration of 44.7 ± 10.5 ppm formic acid for 9 days (worker mortality was evident on the 10th day). Fumigation in the other two formic acid fumigated rooms continued until they reached about the same CT product. The medium-term medium-concentration treatment was 39.4 ± 5.8 ppm formic acid for 10 days and the long-term low-concentration treatment was 19.0 ± 1.5 ppm formic acid for 27 days. Live dissections, as described above, were done on tracheal mite infested bees on 9 January and 7 February 2002.

Experiment 3

On 21 November 2002, 80 honey bee colonies contained in single Langstroth hive bodies were brought indoors where they were placed randomly in stacks in a large holding room (5.2 x 7.2 x 2.5 m). On 10 January 2003, colonies were randomly assigned to one of four experimental treatment rooms. Each hive had a completely open bottom entrance (37 x 2.5 cm) and a small top entrance (5.7 x 1.8 cm). The hives were placed in four stacks of five hives each with the entrances perpendicular to the air distribution tube (Underwood and Currie, 2004).

The four experimental treatment rooms consisted of an untreated control room and three rooms fumigated with a low concentration of formic acid beginning on 18 January 2003. The control room and two of the fumigated rooms had variable ventilation (as described above). The third fumigated room had the ventilation set to a constant minimum (0.4 L/s/m³). One variable ventilation and one minimum ventilation room were fumigated for a total of 47 days. To simulate the effect of a winter warming spell, the heaters in these two rooms were set to 10 °C to raise the temperature for the last six days of fumigation. The third treated room was fumigated for 65 days with the thermostat set to a constant 5 °C. A warm-up occurred naturally as outside temperatures increased to daily maxima of -1 to 13 °C starting on day 55 (14 March) of fumigation and continuing for the duration of the fumigation period.

Statistical Analysis

These experiments were set up as Before-After Control-Impact designs (Stewart-Oaten et al., 1986; Smith, 2002) using the hive as a replicate. A split-plot factorial analysis of variance (PROC GLM; SAS Institute Inc., 1999) was used to analyze the change in nosema spore mean abundance and in tracheal mite prevalence from the beginning to the end of experiment 1. Fumigation level and ventilation level

were main plot factors and time was the sub-plot factor. Changes in pre- and post-treatment level of nosema spore mean abundance and tracheal mite prevalence were assessed using fumigation treatment x time or ventilation treatment x time interactions. Data on nosema spore mean abundance were analyzed after square root transformation. Data on tracheal mite prevalence were analyzed after arcsine transformation. Mite mortality data for live dissections in experiments 1 and 2 were analyzed using PROC CATMOD (SAS Institute Inc., 1999).

Similarly, a split-plot analysis of variance with a BACI design was also used to analyze the change in nosema spore mean abundance and in tracheal mite prevalence from the beginning to the end of the experiments 2 and 3. These tests used treatment room and replicate as main plot factors and time as the subplot factor. Treatment room x replicate was used as the error term for the main effects. Data on nosema spore mean abundance were analyzed after square root transformation. Data on tracheal mite prevalence were analyzed after arcsine transformation.

RESULTS

Tracheal mite prevalence

Tracheal mite prevalence, as measured by thoracic slices, was not affected by formic acid treatment in any of the three experiments, as indicated by non-significant treatment x time interactions (experiment 1; $F = 0.03$, $df = 1, 15$, $P > 0.05$; experiment 2; $F = 1.48$, $df = 3, 42$, $P > 0.05$; experiment 3; $F = 1.83$, $df = 3, 38$, $P > 0.05$; Fig. 6.1). In experiment 1, tracheal mite prevalence was also not affected by ventilation level ($F = 0.03$, $df = 1, 15$, $P > 0.05$; Fig. 6.1).

Tracheal mite survival

The proportion of live tracheal mites in dissections was affected by treatment in both experiments where live dissections were done, as indicated by significant formic acid treatment x time interactions. In experiment 1, tracheal mite survival was affected by formic acid fumigation (Chi-square = 324.79, $P < 0.0001$; Fig. 6.2). Ventilation level also affected tracheal mite survival (Chi-square = 38.40, $P < 0.0001$; Fig. 6.2). In experiment 2, tracheal mite survival was also affected by formic acid fumigation (Chi-square = 14.87, $P < 0.001$; Fig. 6.2). Bees in both the low concentration (Chi-square = 13.43, $P < 0.001$) and the high concentration (Chi-square = 5.85, $P < 0.05$) had greater mite mortality than the control.

Nosema disease

Nosema spore mean abundance was not affected by treatment in any of the three experiments, as indicated by non-significant treatment x time interactions. In experiment 1, nosema spore mean abundance was not affected by either ventilation level ($F = 0.64$, $df = 1, 15$, $P > 0.05$) or formic acid treatment ($F = 1.13$, $df = 1, 15$, $P > 0.05$; Fig. 6.3). Similarly, in experiments 2 and 3, nosema spore mean abundance was not affected by formic acid fumigation (experiment 2; $F = 0.36$, $df = 3, 42$, $P > 0.05$; experiment 3; $F = 0.55$, $df = 3, 38$, $P > 0.05$; Fig. 6.3).

DISCUSSION

Indoor winter fumigation of honey bee colonies with formic acid was effective in killing a high percentage of tracheal mites, but did not significantly reduce the proportion of bees with infested tracheae over the duration of the experiments. Under relatively low or decreasing levels of nosema, fumigation did not significantly suppress the mean abundance of nosema spores relative to the controls. In three

separate fumigation experiments, there was no statistical difference between the build-up or maintenance of nosema spore mean abundance in bees from formic acid fumigated colonies as compared to untreated controls.

Tracheal mites

In all three experiments, there was no significant effect of treatment on tracheal mite prevalence, but the prevalence tended to decrease in formic acid treated colonies relative to controls. We attribute the lack of statistical significance to the lack of sensitivity of the thoracic slice method when bee turnover rates are low. During the winter months in northern climates, the honey bee worker population has very little turnover (Harris, 1980). Workers that are reared in late summer and early fall generally live through the winter and few are replaced until the spring when intensive brood rearing begins again (Nelson, 1995). The bodies of dead mites probably remain in the tracheae even when treatment has been successful. The thoracic slice method (Delfinado-Baker, 1984) does not allow differentiation between living and dead mites. Thus, the value of using the thoracic slice method to determine the efficacy of a winter treatment when worker flight is absent and worker mortality is minimal is questionable.

Analysis of data collected by live dissections, where each individual mite was counted and its status as alive or dead determined (Klymochko, 2001), showed that indoor winter fumigation with formic acid was successful in controlling tracheal mites. Both low and high concentrations of formic acid were effective in our two experiments where live dissections were done. It is clear that concentrations suitable for varroa mite control are effective in killing tracheal mites. However, the minimum concentration x time combination that would provide effective control of tracheal mites could not be determined from our experiment.

Live dissections also showed that room ventilation settings can affect tracheal mite survival, even in the absence of formic acid. When the ventilation was kept at the minimum rate, tracheal mite survival was much higher than when ventilation was allowed to increase to control room temperature. This may be due to differences in humidity in ventilated and unventilated rooms. Increasing the ventilation rate lowers humidity because more water vapor from respiring bees is exhausted from the room (Gruszka, 1998). At the same time, an increase in the ventilation rate lowers room temperature. Because the rate of tracheal mite transfer from one host to another increases with temperature (Bailey, 1958; Pettis, 2001), we expected that mite mortality would be increased at higher temperatures when the mites were more exposed to the environment. This work does not support this hypothesis. More work is required to determine the effect of environmental conditions, such as humidity and temperature, on tracheal mite survival in overwintering honey bee colonies.

Nosema disease

Underwood and Currie (2004) showed that indoor winter fumigation suppresses nosema spore build-up when high concentrations of formic acid are used in conditions where the incidence of the disease increases over the winter. In the experiments presented here, we saw similar trends, but they were not statistically significant.

The discrepancy between this study and previous studies showing that acid treatment is effective against nosema disease (deRuijter and van der Steen, 1989; Underwood and Currie, 2004) has not been fully explained. In our experiments, “natural” infestations of nosema spores were used. In both experiments 1 and 2, a small number of untreated colonies showed large increases in nosema spore mean abundance, but all treated colonies showed, at most, slight increases in nosema spore

mean abundance. In experiment 3, infestations were extremely high at the beginning of the winter and decreased in all treatments, including the control, over the winter. If formic acid treatment acts to suppress outbreaks, it would not be detectable under the conditions that occurred during our experiments.

Perhaps, as was seen with tracheal mites, nosema spores remain in the bodies of bees after they are killed by formic acid treatment, causing inaccurate estimates of infection in spring tests. Honey bees do not usually defecate during indoor wintering as they cannot leave the hive. Therefore, inviable spores present in the gut will be counted in spring examinations of overwintered bees that are conducted before they are able to take a cleansing flight (Pickard and El-Shemy, 1989). Further study is needed to determine whether spores remain viable after formic acid treatment.

This study showed that tracheal mites could be controlled by indoor winter fumigation with formic acid at concentrations suitable for controlling varroa mites (Underwood and Currie, 2004, chapters 3, 4, 5). Winter fumigation with formic acid may help to suppress nosema disease in some situations, but further study with inoculated colonies is required to determine the true efficacy of this treatment method. Mean abundance of nosema spores was not significantly suppressed relative to untreated colonies.

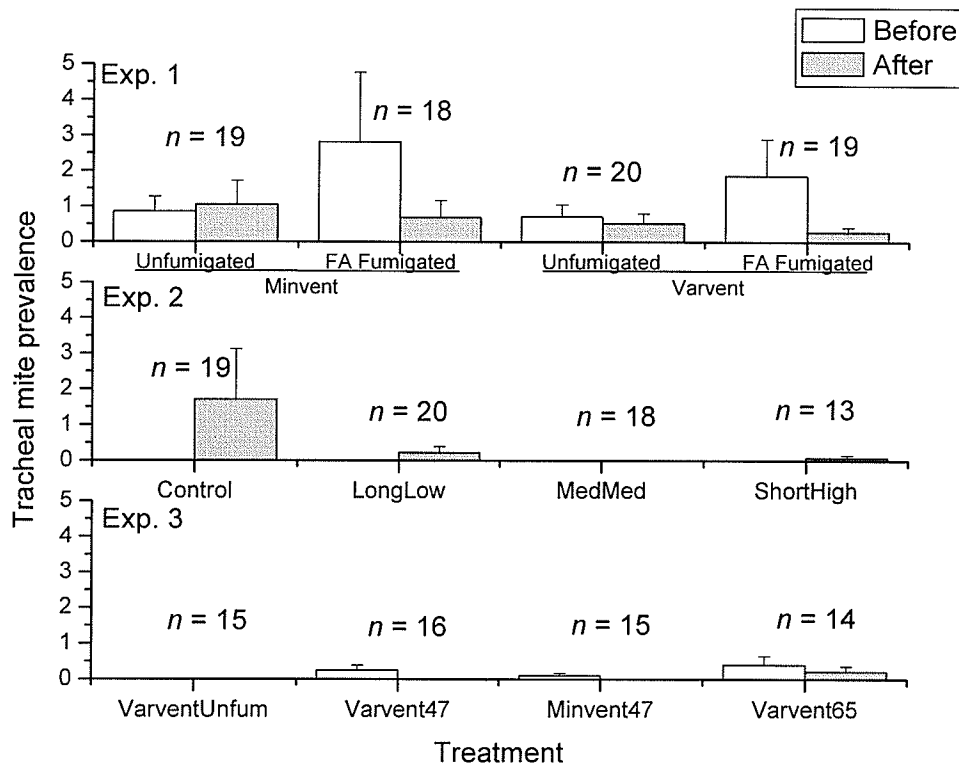


Figure 6.1. Tracheal mite prevalence (mean no. infested bees per 100 bees \pm SE) as measured by thoracic slices before and after formic acid fumigation in three separate indoor winter fumigation experiments. Colonies in experiment 1 (Exp. 1) were unfumigated or treated with a high concentration of formic acid (FA Fumigated) with minimum ventilation (Minvent) or variable ventilation (Varvent). Colonies in experiment 2 (Exp. 2) were untreated (Control), or treated with a long-term low concentration (LongLow), a medium-term medium concentration (MedMed) or a short-term high concentration (ShortHigh) of formic acid, all with minimum ventilation. *n* = number of colonies. Colonies in experiment 3 (Exp. 3) were untreated (VarventUnfum) with variable ventilation, treated with a long-term low concentration with minimum ventilation (Minvent47) or variable ventilation (Varvent47) for 47

days, or with variable ventilation for 65 days (Varvent65). n = number of colonies. No significant treatment x time interactions were found ($P > 0.05$).

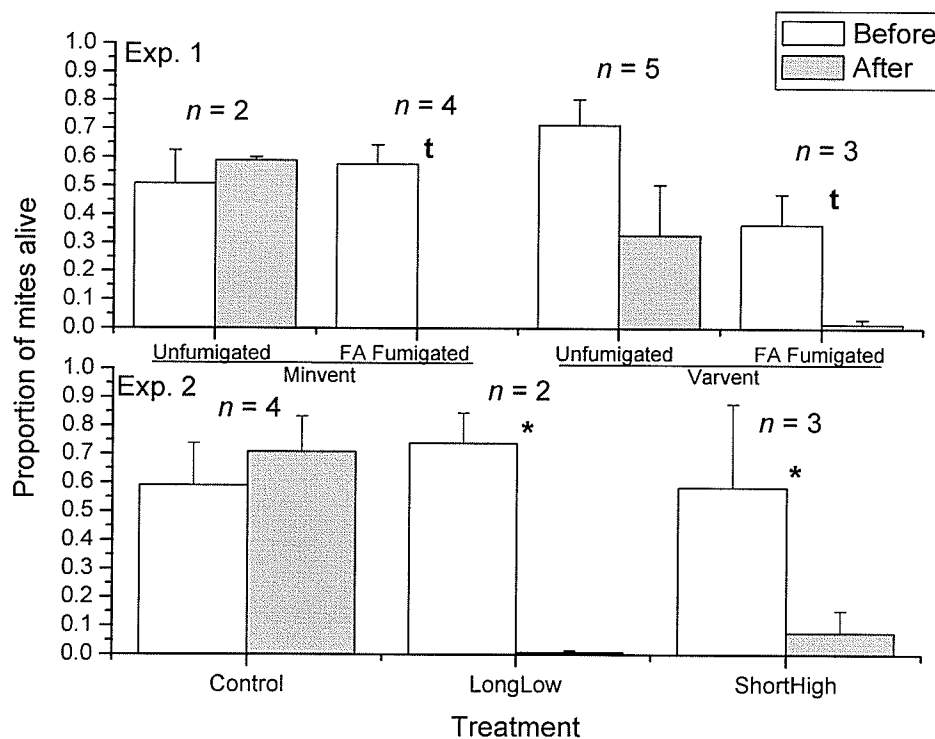


Figure 6.2. Proportion of tracheal mites alive (mean \pm SE) as measured by live dissection before and after formic acid fumigation in two separate indoor winter fumigation experiments. Colonies in experiment 1 (Exp. 1) were unfumigated or treated with a high concentration of formic acid (FA Fumigated) with minimum ventilation (Minvent) or variable ventilation (Varvent). A 't' indicates a significant formic acid treatment x time interaction within ventilation level ($P < 0.0001$). There was also a significant ventilation level x time interaction in colonies in the unfumigated treatment room ($P < 0.01$). Colonies in experiment 2 (Exp. 2) were untreated (Control), or treated with a long-term low concentration (LongLow), a medium-term medium concentration (MedMed) or a short-term high concentration (ShortHigh) of formic acid, all with minimum ventilation. n = number of

colonies. An asterisk indicates a treatment x time interaction that is significantly different from the control ($P < 0.05$).

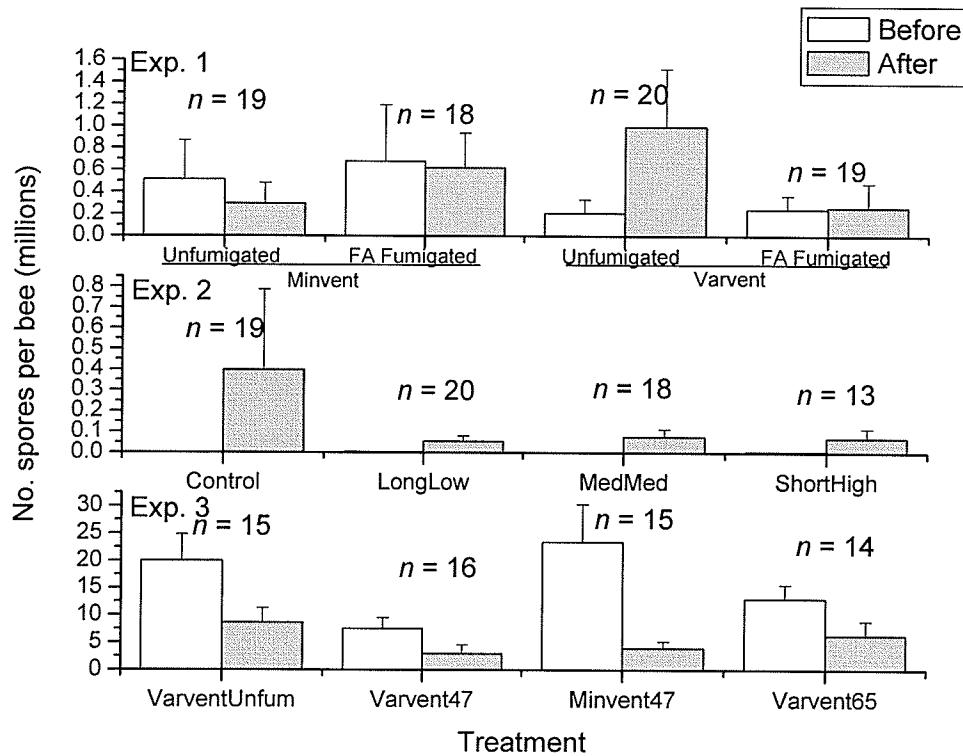


Figure 6.3. Nosema spore mean abundance (mean \pm SE) before and after formic acid

fumigation in three separate indoor winter fumigation experiments. Colonies in experiment 1 (Exp. 1) were unfumigated or treated with a high concentration of formic acid (FA Fumigated) with minimum ventilation (Minvent) or variable ventilation (Varvent). Colonies in experiment 2 (Exp. 2) were untreated (Control), or treated with a long-term low concentration (LongLow), a medium-term medium concentration (MedMed) or a short-term high concentration (ShortHigh) of formic acid, all with minimum ventilation. n = number of colonies. Colonies in experiment 3 (Exp. 3) were untreated (VarventUnfum) with variable ventilation, treated with a long-term low concentration with minimum ventilation (Minvent47) or variable ventilation (Varvent47) for 47 days, or with variable ventilation for 65 days (Varvent65).

n = number of colonies. No significant treatment x time interactions were found ($P > 0.05$).

CHAPTER 7. INDOOR WINTER FUMIGATION WITH FORMIC ACID DOES NOT HAVE A LONG-TERM EFFECT ON HONEY BEE QUEEN PERFORMANCE

ABSTRACT

Formic acid fumigation has been used to control infestations of the varroa mite, *Varroa destructor* Anderson and Trueman, and the tracheal mite, *Acarapis woodi* (Rennie) in various situations. Studies on the use of formic acid in honey bee colonies have generally focused on the immediate, direct effects of treatment. These immediate effects of treatment are important to consider, but the potential for long-term effects are also of concern to beekeepers. The objective of this study was to determine whether indoor winter fumigation of honey bee colonies with formic acid affects long-term honey bee queen performance by measuring sealed brood production, the frequency of queen supersedure, and honey production.

Two experiments, during which honey bee colonies were fumigated with formic acid in an indoor wintering facility, were conducted during the winter of 2001 – 2002. During the subsequent summer, winter-fumigated colonies with their original queens were evaluated for brood and honey production and queen supersedure after equalization of varroa mite and honey bee populations.

Performance of queens that had been fumigated with a variety of different formic acid concentration-exposure time combinations during the winter did not differ from unfumigated queens in terms of brood production, supersedure rates or honey production. Previous studies have shown that indoor winter fumigation can be safely administered to honey bee colonies without causing immediate harm to workers or

queens. This study shows that this treatment also lacks long-term effects on queen honey bees. Whether a long-term low concentration or a short-term high concentration was used, surviving queens were as productive as untreated wintered queens.

INTRODUCTION

Formic acid fumigation has been used to control infestations of the varroa mite, *Varroa destructor* Anderson and Trueman, and the tracheal mite, *Acarapis woodi* (Rennie) in various situations (e.g. Hoppe et al., 1989; Liu and Nasr, 1992; Nelson et al., 1994; Calis et al., 1998; Calderone and Nasr, 1999; Ostermann and Currie, 2004; Underwood and Currie, 2004). Studies on negative effects of formic acid in honey bee colonies have generally focused on the immediate, direct effects of treatment. For example, when formic acid is applied in June to colonies with mite populations below the economic threshold, the treatment reduces brood production and worker bee population size (Ostermann and Currie, 2004). Brood can be harmed during fumigation (Fries, 1991; Bolli et al., 1993), but the harm tends to only be to brood in close proximity to the site of the formic acid and affected colonies quickly recover (Hoppe et al., 1989; Westcott and Winston, 1999). Other studies have shown no effect of formic acid on emergence weights of worker bees or foraging activity of treated workers (Sharma et al., 1983; Liu and Nasr, 1992). During indoor winter treatments, worker and queen mortality can be increased by formic acid treatment, but only when high concentrations of acid are used (Underwood and Currie, 2003, 2004, chapters 3, 4, 5). These immediate effects of treatment are important to consider, but the potential for long-term effects is also of concern to beekeepers.

Because of the high turnover of workers during the summer months and the relatively long lifespan of queens, studies of the long-term effects of formic acid fumigation should focus specifically on the effect of treatment on queens and their performance. Westcott and Winston (1999) and Nelson et al. (1994) measured colony weight gain and population growth in colonies during the summer after spring treatment with formic acid. They found that these variables did not vary with

treatment, showing that there are no long-term effects of spring formic acid treatments on long-term queen performance. However, the potential for sublethal effects of indoor winter treatments on queen performance has not been examined (Underwood and Currie, 2004, chapters 3, 4, 5). The objective of this study was to determine whether indoor winter fumigation of honey bee colonies with formic acid affects long-term honey bee queen performance by measuring sealed brood production, the frequency of queen supersedure, and honey production.

MATERIALS AND METHODS

Two experiments, during which honey bee colonies were fumigated with formic acid in the indoor wintering facility at the University of Manitoba, in Winnipeg, Manitoba, Canada (N 49° 48' 32", W 97° 07' 37") were conducted during the winter of 2001 - 2002 (chapters 3, 4). These colonies were moved outdoors on 8 April 2002 and divided between two apiary sites east of Windygates, Manitoba, Canada (N 49° 05', W 98° 06') where they remained for the duration of the summer. Hive entrances faced in different directions and boxes of different colors were used to reduce drift between hives (Jay, 1966; Jay and Dixon, 1988; Taber, 2002).

Experiment 1

Indoor winter formic acid fumigation treatments consisted of four fumigation levels. Each treatment room contained 21 full-size colonies held in single Langstroth hive bodies. One room, the control, remained unfumigated, with no formic acid exposure. The other three rooms were fumigated with formic acid using different treatment regimes. Starting on 11 January, treated colonies were exposed to a low, medium, or high concentration of formic acid until a concentration x time (CT) product of approximately 390 ppm*days was realized in room air in each

experimental room. 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 390 ppm*days. The short-term high-concentration treatment consisted of a room air concentration of 44.7 ± 10.5 ppm formic acid (in-hive; 12.3 ± 3.1 ppm) for 9 days (worker mortality was evident on the 10th day). The other two formic acid fumigated rooms then continued until they reached a similar CT product. The medium-term medium-concentration treatment was 39.4 ± 5.8 ppm formic acid (in-hive; 15.5 ± 3.4 ppm) for 10 days and the long-term low-concentration treatment was 19.0 ± 1.5 ppm formic acid (in-hive; 6.0 ± 0.4 ppm) for 27 days. During the summer, the colonies were kept in a two apiary sites located within 2 km of each other. See chapter 4 for a detailed account of the experiment.

Experiment 2

Indoor winter formic acid treatments consisted of two fumigation levels; fumigated and unfumigated and two ventilation levels; minimum and variable. During fumigation, one treatment pair had only the minimum ventilation (0.4 L/s/m^3) while the other pair had variable ventilation, where the minimum ventilation was supplemented with an additional fan set on a thermostat that increased the ventilation rate to 8.2 L/s/m^3 when room temperature rose above 6°C . Each treatment room contained 21 full-size honey bee colonies held in single Langstroth hive bodies. Fumigation began on 4 March 2002 and the acid was removed after 7 days of fumigation on 11 March. Colonies were exposed to an average formic acid concentration of 27.1 ± 1.1 ppm in room air (in-hive; 6.3 ± 0.8 ppm) in the variable ventilation treatment room and 56.9 ± 8.1 ppm in room air (in-hive; 14.1 ± 2.2 ppm)

in the minimum ventilation treatment room. During the summer, all colonies were kept in a single apiary. See chapter 3 for a detailed account of the experiment.

Determination of long-term effects

All colonies were examined on 17 and 30 April to determine which were queenright by the direct observation of eggs and/or the queen. All surviving queens were marked with paint and the color was recorded. A Bee Boost queen pheromone lure (Phero Tech Inc., Delta, British Columbia, Canada) was placed in colonies that lost queens during the winter on 3 April and remained in place until queens became available. These and additional queenless colonies as detected on 17 April were then successfully requeened, but eliminated from this study as the queens would not have been overwintered or subjected to formic acid treatment.

On 8 April, all colonies were treated with Apistan according to label recommendations to eliminate varroa mites prior to the determination of long-term effects of formic acid fumigation on queens. For colonies from experiment 1, the Apistan was replaced with Coumaphos according to label recommendations on 14 May because some of these colonies contained Apistan-resistant varroa mites according to the Pettis test (Pettis et al., 1998). Coumaphos could not be used before that date as emergency registration was not yet approved. On 5 June, the honey bee worker populations were equalized for bees and brood so that each contained approximately 6 frames of bees (approximately 12,000 bees). On 10 June, all acaricide strips were removed from the colonies to allow the appropriate withdrawal period prior to the honey flow. Capped brood area was estimated for colonies in experiment 1 on 26 or 28 June and then again on 8 or 10 July using a piece of plexiglass the size of a frame marked with a 1 cm grid (Rogers et al., 1983).

Honey supers were added as needed throughout the summer. On 14 August, all honey supers were removed and weighed prior to and after honey extraction. Queen status was assessed during brood sampling and in a final inspection on 25 September to assess supersedure frequency. A colony containing no queen or an unmarked queen upon final inspection in the fall was considered to have experienced a supersedure. One colony from the control group died during the evaluation period and was eliminated from the experiment.

Statistical Analysis

The effect of treatment on honey production was assessed using an analysis of variance (ANOVA) after log transformation (PROC GLM; SAS Institute Inc., 1999). For experiment 1, a randomized block design was used to assess the effect of formic acid fumigation regime. For experiment 2, a factorial design was used to assess the effects of fumigation and ventilation treatment levels.

The effect of treatment on the area of capped brood on each sampling date in colonies in experiment 1 was analyzed using ANOVA (PROC GLM; SAS Institute Inc., 1999). The effect of treatment on the frequency of queen supersedure in colonies from each experiment was analyzed using Fisher's exact test (PROC FREQ; SAS Institute Inc., 1999).

RESULTS

In experiment 1, brood production of queens fumigated with formic acid during the previous winter did not differ from unfumigated queens on 26 or 28 June ($F = 0.70$, $df = 3, 37$, $P > 0.05$) or 8 or 10 July ($F = 0.87$, $df = 3, 37$, $P > 0.05$; Fig. 7.1).

In experiment 1, honey production in colonies with formic acid-fumigated queens did not differ from those with unfumigated queens ($F = 2.63$, $df = 3, 17$, $P = 0.08$; Fig. 7.2). Similarly, in experiment 2, honey production was not affected by ventilation level or fumigation treatment (ventilation; $F = 1.73$, $df = 1, 36$, $P > 0.05$; fumigation; $F = 0.03$, $df = 1, 36$, $P > 0.05$; Fig. 7.2).

The frequency of queen supersedure also was not affected by formic acid fumigation (experiment 1; $P > 0.05$; experiment 2; $P > 0.05$). In experiment 1, queen supersedure occurred in two of 15 colonies in the unfumigated group, three of 14 in the long-term low concentration group, five of 10 in the medium-term medium concentration group and zero of four in the short-term high concentration group. In experiment 2, queen supersedure occurred in nine of 20 colonies in the unfumigated minimum ventilation group, one of nine in the fumigated minimum ventilation group, in six of 15 the unfumigated variable ventilation group, and four of 15 in the fumigated variable ventilation group. Overall, 17 of 50 (34 %) unfumigated queens were superseded while 13 of 52 (25 %) formic acid fumigated queens were superseded, but these rates were not significantly different from each other ($P > 0.05$).

DISCUSSION

Performance of queens fumigated with a variety of different formic acid concentration-exposure time combinations during the winter did not differ from unfumigated queens in terms of brood production, supersedure rates, or honey production. Previous studies have shown that winter fumigation with formic acid can be safely administered to honey bee colonies without causing immediate harm to workers or queens under some combinations of concentration and exposure time (Underwood and Currie, 2004, chapters 3, 4, 5). This study shows that this treatment

also lacks long-term effects on surviving queen honey bees. Whether a long-term low concentration or a short-term high concentration was used, surviving queens were as vigorous and productive as unfumigated wintered queens.

In our experiment, all colonies were treated with Apistan and/or Coumaphos at the beginning of the observation period to remove varroa mites. Therefore, the results indicate the true long-term effects of the winter treatments without confounding treatment effects of formic acid with treatment effects caused by varroa mite populations. In the absence of varroa mites, formic acid treatments applied during the spring can have a detrimental effect on brood production (Ostermann and Currie, 2004), but this is likely due to a direct effect of the acid on brood survival (Fries, 1991; Bolli et al., 1993). Brood rearing in spring is critical to the success of the colony over the summer, affecting honey production directly (Harbo, 1986). Formic acid fumigation can also negatively effect drone production and survival (DeGuzman et al., 1999). The results of our study show that winter fumigation with formic acid would not affect brood production in spring as long as honey bee population sizes were not also reduced by treatment.

During fumigation, honey can absorb formic acid (e.g. Stoya et al., 1986; Wachendörfer and Keding, 1988; Liu et al., 1993; Bogdanov et al., 2002; chapter 5), requiring bees to consume food stores with elevated formic acid concentrations during and after fumigation. Studies have shown that concentrations return to pre-treatment levels after 12 - 38 weeks (Stoya et al., 1986; Wachendörfer and Keding, 1988). The lack of long-term effects on queen performance seen in this study suggests that any sublethal effects caused by indirect effects associated with the consumption of acid feed do not affect the queen's productivity during the summer.

This study showed that there is no long-term effect of formic acid fumigation on the performance of surviving queens as a result of treatment indoors in winter. Neither the amount of brood produced, the amount of honey produced, nor the frequency of queen supersedure was affected by winter treatments. The results showed that formic acid fumigation during the winter months did not have long-term consequences for colony population growth or queen survival through the following production season.

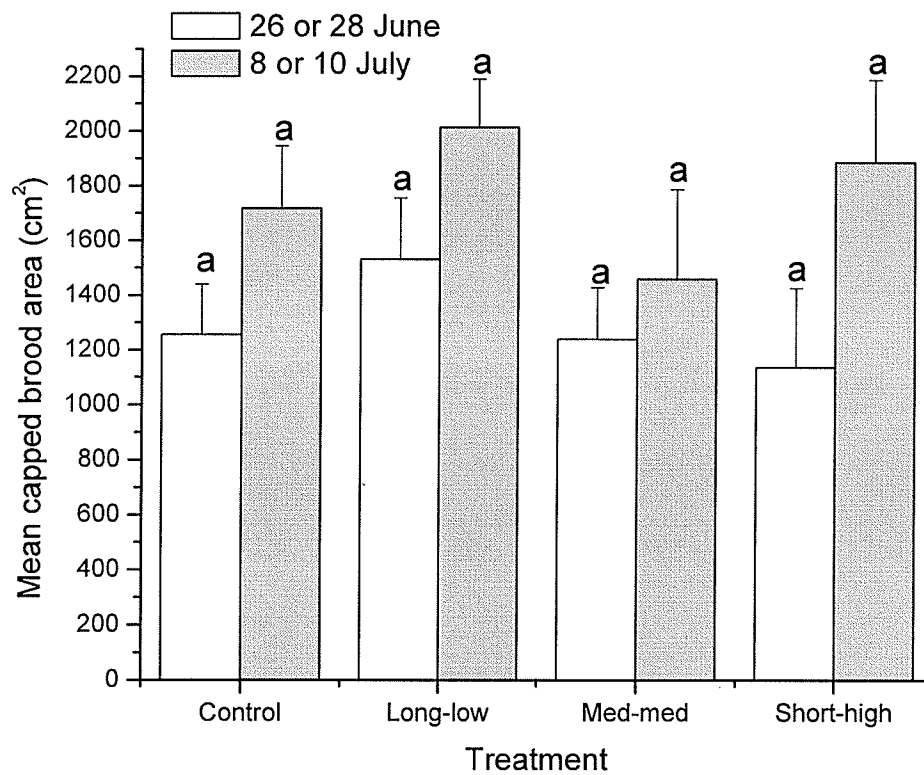


Figure 7.1. Capped brood area (mean \pm SE) for colonies unfumigated (Control; $n = 14$) or treated with formic acid using a long-term low concentration (Long-low; $n = 14$), medium-term medium concentration (Med-med; $n = 8$) or short-term high concentration (Short-high; $n = 4$) during the winter of 2001-2002 in experiment 1. Columns with the same letter are not significantly different from the other columns within that sampling date ($P > 0.05$).

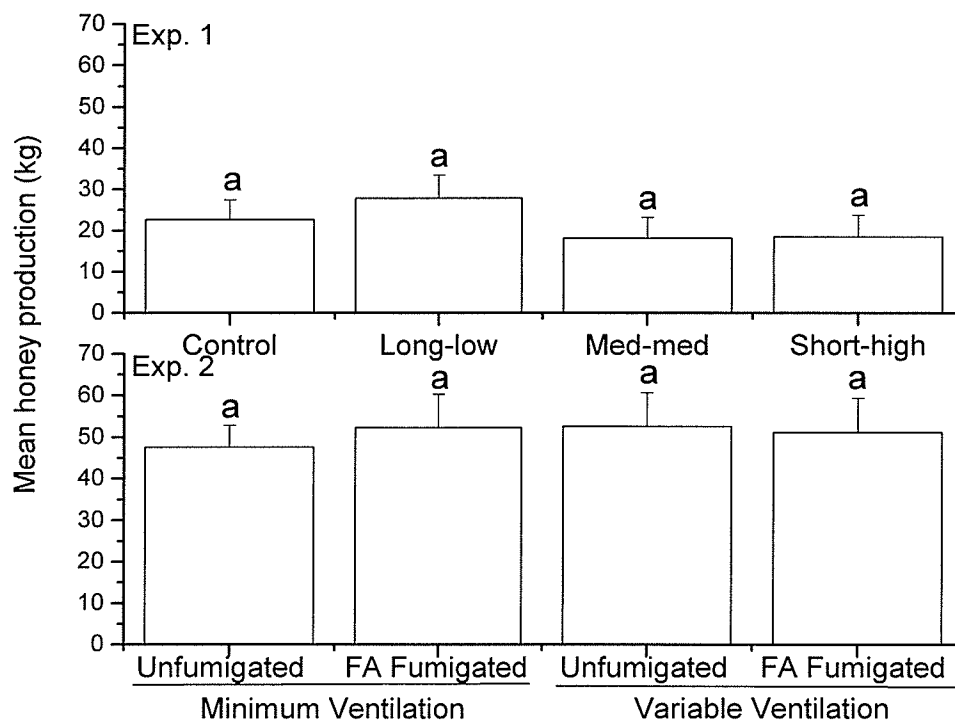


Figure 7.2. Honey production (mean \pm SE) during the summer of 2002 for colonies untreated or treated with formic acid during the winter of 2001-2002. In experiment 1 (Exp. 1), experimental treatments were untreated (Control; $n = 15$), a long-term low concentration (Long-low; $n = 14$), a medium-term medium concentration (Med-med; $n = 10$) and a short-term high concentration (Short-high; $n = 4$). In experiment 2 (Exp. 2), experimental treatments were unfumigated with minimum ventilation ($n = 20$), formic acid-fumigated (FA Fumigated) with minimum ventilation ($n = 9$), unfumigated with variable ventilation ($n = 15$), and formic acid-fumigated with variable ventilation ($n = 15$). Bars with the same letter are not significantly different from the other columns within that experiment ($P > 0.05$). Mite and bee populations were equalized at the start of the season.

CHAPTER 8. FORMIC ACID IN BEE FEED: ITS EFFECT ON HONEY BEE QUEEN PHEROMONES AND ATTENDANCE BY WORKERS.

ABSTRACT

Beekeepers manage honey bees, *Apis mellifera* L., for crop pollination and honey production and use chemicals to maintain active, healthy colonies that are disease and pest free. Formic acid is applied as a fumigant in colonies to control the varroa mite (*Varroa destructor* Anderson and Trueman). When this treatment has been applied indoors in winter, substantial queen losses have been observed. One potential source of the problem is the formic acid that is absorbed by the bees' food stores. If the queen is fed acid-laden sugar syrup or honey by worker attendants, her ability to produce the complex blend of pheromones necessary to maintain a healthy colony may be affected. This study was conducted to determine whether acid-laden sugar syrup affects the attractiveness of queens to attendant workers, the quantity of her pheromone blend, or her mortality rate.

Formic acid in sugar syrup reduced the proportion of worker bees attending the queen in summer bees, but not in winter bees. Bees fed 100 ppm formic acid in sugar syrup for 37 or 38 days were less likely to attend the queen than bees fed unadulterated sugar syrup while bees fed 1,000 ppm formic acid in syrup did not differ from the control or 100 ppm treatment. Formic acid did not affect the level of worker attendance in winter bees. However, there was a seasonal difference in the quantity of one pheromone component, methyl-*p*-hydroxybenzoate, in the body wash. Neither treatment nor time of year of the trial affected the quantity of any other

pheromone component in either the queen's mandibular glands or in solvent washes of her body. No queen mortality was observed.

INTRODUCTION

Honey bees, *Apis mellifera* L., are eusocial insects that use pheromones for communication. In particular, the queen produces a complex pheromone blend that serves to maintain colony unity, prevent worker ovary development (Jay, 1968), influence colony reproduction (Winston et al., 1989; Pettis et al., 1995) and attract workers to her retinue (Gary, 1961; Slessor et al., 1988). Workers in the retinue antennate and lick the queen and, subsequently, disperse the pheromone to the other individuals in the colony (Seeley, 1979; Slessor et al., 1988, 1990; Naumann et al., 1991, 1992). Eight major components of the queen pheromone complex are responsible for the majority of the retinue response of worker bees. These are 4-hydroxy-3-methoxyphenylethanol (hva), methyl *p*-hydroxybenzoate (hob), 4-hydroxy-3-methoxycinnamyl alcohol or coniferyl alcohol (coni), (E)-9-oxodec-2-enoic acid (9-oda), (E)-9-hydroxydec-2-enoic acid (9-hda), methyl oleate (meo), linolenic acid (lino), and hexadecanol (hex) (Callow and Johnston, 1960; Barbier and Lederer, 1960; Callow et al., 1964; Butler and Fairey, 1964; Slessor et al., 1988; Kaminski et al., 1990; Keeling et al., 2003). These pheromones are produced in the queen's mandibular glands, cephalic labial gland, and tergal gland (Barbier and Lederer, 1960; Callow and Johnston, 1960; Slessor et al., 1988; Wossler and Crewe, 1999; Keeling et al., 2003).

Beekeepers manage honey bees for crop pollination and honey production and use natural and synthetic chemicals to maintain active, healthy colonies that are disease and pest free. One such chemical is formic acid, which when applied as a fumigant in honey bee colonies to control the varroa mite (*Varroa destructor* Anderson and Trueman), can cause substantial queen loss (Underwood and Currie, 2004, chapters 3, 4). The exact cause of these losses has yet to be determined, but

could be due to sublethal concentrations of formic acid that cause disruption of queen pheromone production. Formic acid is absorbed by the bees' food stores (e.g. Stoya et al., 1986; Hansen and Guldborg, 1988; Liu et al., 1993; chapter 5) and acid-laden sugar syrup or honey fed to her by worker attendants could affect her ability to produce the complex blend of pheromones. A decrease in the queen's pheromone level could then lead to queen mortality and replacement through supercedure (Butler, 1960). This study was conducted to determine whether acid-laden sugar syrup fed to worker attendants affects the attractiveness of queens to worker attendants, the quantity of her pheromone blend, or her mortality rate. Two trials, one in summer and one in winter, were conducted because of the possible effect of changes in worker physiology and queen attractiveness with season.

MATERIALS AND METHODS

Bioassay

All queens used in this study were reared from randomly-selected colonies of New Zealand stock during the summer of 2002 and allowed to "open mate" with the local drone population in late July. For the summer trial, queens were free running in nuclear colonies until they were caged for the bioassay. In October, all remaining queens were caged and placed in a queen bank in a single, queenless colony, where they remained until the winter bioassay began.

Bioassays were conducted with mixed-age workers in 10 x 7 x 5 cm plexiglass cages (Fig. 8.1) covered with metal hardware cloth (2.3 squares per cm). The top of the cage had a 2.5 cm hole over which a 50 mL centrifuge feeder tube was placed. The lid of the tube had a 1.6 cm diameter hole cut into it that was covered with a fine mesh straining cloth (24.6 squares per cm). The queen was confined to a 4.1 x 4.3 x

2.1 cm wooden queen cage with one large side open and two small sides made of clear plastic for viewing purposes. The open side of the queen cage was held against the cage containing the workers by a thin wire. The workers could antennate, lick and feed the queen through the metal screen (Fig. 8.1). Cages were held at 30 °C.

The feeders contained a 67 % sugar solution (as measured by a refractometer) of a 45:55 glucose:fructose ratio in distilled water. The three treatments consisted of syrup with 0, 100, or 1,000 ppm formic acid.

For each trial, workers were collected from a single queenless colony, anesthetized with carbon dioxide for 2 min and counted into cages. Each cage received 35 workers and the cages were randomly assigned to the three treatments. To prevent results being influenced by worker mortality, which can occur with high concentrations of acid (Currie, unpublished), workers were replaced every second day. Feeders were also replaced on the same schedule. Once workers were in cages, they were starved for 1-3 h. They were then given the syrup and allowed to feed for at least 2 hours before the queen was placed on the cage. The worker source colony was maintained outdoors until early November when it was brought indoors and maintained at 5 °C.

The proportion of workers in contact with the queen cage or not in contact with the queen cage was counted daily for 37 (summer) and 38 days (winter). The experiment was repeated in the summer (from 17 Aug until 23 September; $n = 6$) and winter (from 4 November until 11 December 2002; $n = 2$).

Pheromone analysis

At the end of each trial, all queens were subjected to a body wash and the mandibular glands were dissected for pheromone quantification according to the methods of Naumann et al. (1991) as follows. The queen was anesthetized with

carbon dioxide in a sample cup for 30 s. Body washes of pheromone were obtained by holding the antennae with clean forceps and dribbling 300 μL reagent grade methanol over her head. The solvent was collected in a 1 mL glass vial from the tip of her abdomen. The queen was then pinned to a wax dissecting dish with a paper collar to hold her head in place. Both mandibular glands were removed and crushed in 100 μL reagent grade methanol. The body wash and gland samples were held at -20 °C until all queens were dissected and transferred to -80 °C for storage within 1.5 h.

For body wash analysis, 100 μL of the extract plus 1 μL of an internal standard containing 1.67 μg of decanoic acid were placed in a glass vial. The solvent was evaporated out by exposing the sample to reduced pressure. The extract was then resuspended in pentane. Granulated drying agent was added and the solvent was again evaporated out by blowing nitrogen over the surface while continuously rinsing down the sides of the vial. The extract was then resuspended in hexane and transferred to a vial with a 100 μL capacity insert. The solvent was evaporated out under reduced pressure. Trimethylsilyl derivatives of the compounds were then created by adding 2 μL bis(trimethylsilyl)trifluoroacetamide (BSTFA) (T-1506, Sigma-Aldrich Co., St. Louis, MO, USA) and incubating the sample at room temperature for 40 min. The derivatives were then resuspended in 20 μL hexane of which 3 μL were injected into the gas chromatograph-mass spectrometer (GC-MS; HP5890, Hewlett-Packard Company, Palo Alto, CA) for quantification.

For the glands, 4 μL of the extract plus 1 μL of an internal standard containing 5 μg of decanoic acid were placed in a glass vial with 100 μL capacity insert. The solvent was then removed under reduced pressure. Trimethylsilyl derivatives were

created as described above and 100 μ L hexane was added to resuspend the pheromone extract of which 2 μ L was injected into the gas chromatograph for quantification.

Pheromone analysis followed Slessor et al. (1990) and Keeling et al. (2003) with modifications. For quantitative analysis of each component of the pheromone complex in the body washes, selected ion monitoring was used in splitless mode on a 30 m DB-1 column in the GC-MS (60 °C for 1 min, 10 °C/min to 220 °C, isothermal for 5 min). The internal standard, decanoic acid, was detected using 129, 117, and 229 (10.5-11.4 min retention time); hob 135, 209, 224 (11.4-13.5 min); 9-oda and hva 209, 312, 179, 166, 155, 81, 55 (13.5-14.9 min); 9-hda 147, 117, 81 (14.9-16.0 min); conl and hexa 204, 293, 324, 299, 75, 300 (16-18 min); meo 131, 55, 84, 74 (18-19.5 min) and lino 335, 129, 75, 67 (19.5-22 min). For analysis of mandibular gland components, total ion monitoring (50-550) was used. The same injector, detector and oven temperatures were used as for washes.

To determine the quantity of each pheromone component in each sample, commercial standards were derivatized with BSTFA, as above, and used to determine the response of the instrument with respect to the internal standard. These were done using selected ion monitoring for use with the body wash samples and total ion monitoring for use with the gland samples.

Statistical analysis

The effect of sugar syrup treatment on the proportion of worker bees attending the queen in each trial over time was determined by a repeated measures analysis of variance (ANOVA) using an autoregressive heterogeneous covariance structure after arcsine transformation (PROC MIXED; SAS Institute Inc., 1999). When significant treatment effects were observed within a season, treatment means were compared using contrasts with Bonferroni-corrected alpha values.

The effect of syrup treatment and season on the quantity of individual pheromone components in the body wash and gland samples in each trial was determined by ANOVA (PROC GLM; SAS Institute Inc., 1999).

RESULTS

Bioassay

During the summer trial, there was a significant effect of treatment on the proportion of workers attending the queen ($F = 4.25$, $df = 2, 15$, $P < 0.05$; Fig. 8.2). Fewer workers in the low concentration treatment attended the queen than in the control ($F = 8.18$, $df = 1, 15$, $P = 0.01$). The proportion of workers attending the queen in the high concentration treatment was not significantly different from that in the control or low concentration treatments ($F = 3.69$, $df = 1, 15$, $P > 0.05$). This effect did not change over time as indicated by a nonsignificant treatment x time effect ($F = 1.12$, $df = 72, 537$, $P > 0.05$). During the winter trial, there was no significant effect of treatment on the proportion of workers attending the queen ($F = 2.03$, $df = 2, 3$, $P > 0.05$; Fig. 8.2), but the proportion varied sporadically with time ($F = 1.95$, $df = 74, 111$, $P < 0.001$).

Pheromone analysis

Formic acid in sugar syrup did not affect the quantity of any individual pheromone component in the body washes in either summer or winter (Table 8.1; Fig. 8.3). Season affected the quantity of hob in body washes, but did not affect the quantity of any other pheromone component (Table 8.1; Fig. 8.4). Hob was detectable only in the summer.

Similarly, sugar syrup treatment did not affect the quantity of any individual pheromone component in the queen mandibular glands in either the summer or winter

trial (Table 8.2; Fig. 8.4). Treatment season did not affect the quantity of any pheromone component (Table 8.2).

DISCUSSION

Formic acid in sugar syrup affected the proportion of worker bees attending the queen in summer bees, but not in winter bees. Bees fed 100 ppm formic acid in sugar syrup were less likely to attend the queen than bees fed unadulterated sugar syrup while bees fed 1,000 ppm formic acid in syrup did not differ from the control or 100 ppm treatment. Formic acid did not affect the level of worker attendance in winter bees, but there was a seasonal difference in the level of one pheromone component (hob) in the body wash. Neither treatment nor time of year of the trial affected the quantity of any other pheromone component in either the queen's mandibular glands or in solvent washes of her body. No queen mortality was observed.

The difference in results between the summer and winter bioassays may be due to seasonal changes in honey bee physiology. Juvenile hormone titers, hemolymph protein and vitellogenin titers (Fluri et al., 1982; Huang and Robinson, 1995) and worker tolerance of acidity in food (Currie, unpublished) are all different in "winter"- than in "summer"-adapted bees. In addition, attendance of queens by workers changes with the queen's egg-laying frequency, with the queen receiving less attention when she is laying fewer eggs (Perepelova, 1928 in Free, 1959; Allen, 1960) because worker response to queen pheromone declines (Pankiw et al., 1994). Our summer bioassay was conducted when brood rearing was underway in the colonies and queens were free running. Queens in our winter bioassay were banked in a queenless colony for one month prior to the assay and, although they were unable to

lay eggs, they were tested at a time of year when egg-laying is minimal. Slessor et al. (1990) found that banking does not affect queen pheromone quantity, which agrees with our results. However, we did observe a seasonal change in the quantity of one component of the queen's pheromone complex.

Some components of the queen pheromone complex, 9-oda and 9-hda, have been shown to change with season (Pain et al., 1974), but these components did not decline in our study. However, we did see an effect of season on the quantity of hob, which was lower in the winter than in the summer. This has not been reported previously. Further work is necessary to determine whether this pheromone component changes consistently with season. Seasonal differences in the workers' response to queen pheromone and/or composition of the pheromone complex may explain why queens are more susceptible to formic acid in winter than in summer, but this does not appear to be related to a sublethal cumulative effect of formic acid in the food supplied by winter attendants.

The presence of formic acid in sugar syrup failed to affect the quantity or quality of pheromones produced by the queens. Therefore, a lack of care by workers during formic acid fumigation due to a disruption in pheromone production is unlikely to be the cause of her mortality. Westcott and Winston (1999) showed that formic acid fumigation does not affect the number of workers attending queens within one hour after treatment during summer. Further work is necessary to determine the cause of queen loss during formic acid fumigation in winter and should focus on the direct effects of formic acid vapors on queens.

The quantities of pheromone components found in queen body washes in this study were approximately 100x greater than those reported previously (Naumann et al., 1991; Keeling et al., 2003), however, this may be due to the conditions of our

bioassay. In a normal colony situation, workers distribute the queen's pheromones by licking and antennating her while in the retinue (Naumann et al., 1991; Keeling et al., 2003). During this laboratory study, the workers' ability to antennate and lick the queen was somewhat restricted because she was held in a cage with wire screen between her and the workers. Under these conditions, the maintenance of pheromone production could have resulted in a build-up of pheromones on the surface of her body. Naumann et al. (1991) found that, when queens were held in isolation for up to 12 hours, the quantity of 9-ODA in the queen's mandibular glands increased significantly, but this was not reflected in body washes of the queen's body. During our study, queens were in contact with workers, but separated from them by wire screen for 38 days, an extended period of time that may have resulted in an accumulation of pheromone on the queens' bodies. The quantity of pheromones in the gland samples did not differ from those reported in other studies (Pain et al., 1974; Slessor et al., 1990; Pankiw et al., 1996; Keeling et al., 2003) suggesting that pheromone production was not affected by our methodology.

The number of workers attending the queen was influenced by the amount of formic acid in sugar syrup in the summer, but not in the winter. This effect was not reflected in a change in quantity of eight pheromone components on the queen's body or in four pheromone components in her mandibular glands. However, the quantity of one component, HOB, in the queen body wash was lower in winter than in summer. No queen mortality was observed in any treatment. Formic acid in bee feed is not likely the cause of the queen loss that is observed during formic acid fumigation in winter.

Table 8.1. Statistical analysis of pheromone gland quantity in body washes of queens in a bioassay where bees were fed unadulterated sugar syrup (control) or sugar syrup containing 100 ppm (low) or 1,000 ppm (high) formic acid.

Pheromone	Effect of treatment			Effect of trial season			Treatment*trial effect		
	F	df	P	F	df	P	F	df	P
hob	0.16	2,18	0.85	5.75	1,18	0.03*	0.16	2,18	0.85
9-oda	0.12	2,18	0.89	0.46	1,18	0.50	0.64	2,18	0.54
hva	0.10	2,18	0.90	3.17	1,18	0.09	0.10	2,18	0.91
9-hda	0.34	2,18	0.71	0.01	1,18	0.93	0.24	2,18	0.79
coni	0.10	2,18	0.90	1.95	1,18	0.18	0.62	2,18	0.55
hex	0.14	2,18	0.87	0	1,18	0.97	0.54	2,18	0.59
meo	0.08	2,18	0.92	0.34	1,18	0.57	0.23	2,18	0.80
lino	0.44	2,18	0.65	0.74	1,18	0.40	0.29	2,18	0.75

Table 8.2. Statistical analysis of pheromone gland quantity in the mandibular glands of queens in a bioassay where bees were fed unadulterated sugar syrup (control) or sugar syrup containing 100 ppm (low) or 1,000 ppm (high) formic acid.

Pheromone	Effect of treatment			Effect of trial season			Treatment*trial effect		
	F	df	P	F	df	P	F	df	P
hob	0.40	2,18	0.68	0.77	1,18	0.39	0.01	2,18	0.99
9-oda	0.16	2,18	0.86	0.65	1,18	0.43	0.18	2,18	0.83
hva	0.57	2,18	0.58	0.09	1,18	0.77	0.06	2,18	0.95
9-hda	0.31	2,18	0.74	0.63	1,18	0.44	0.14	2,18	0.87

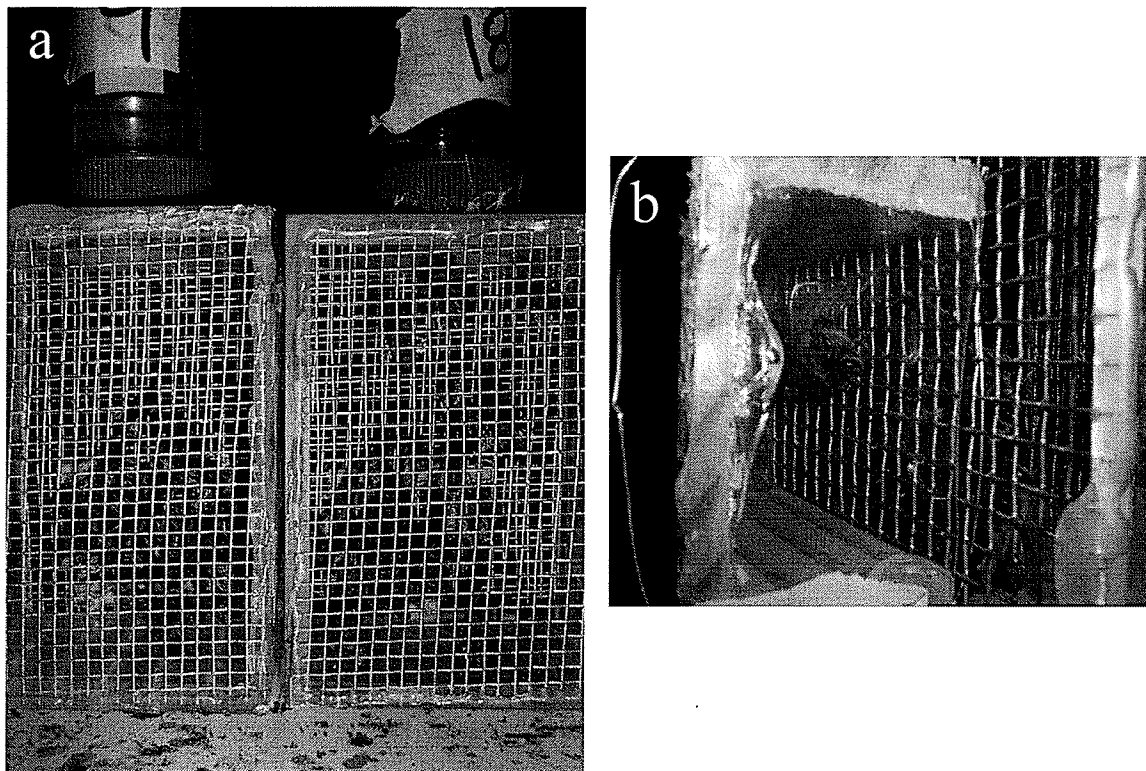


Figure 8.1. Set-up of bioassay cages. Workers were held in a cage (a) and allowed free access to the sugar syrup, while the queen was enclosed in a second, smaller cage (b) that allowed her to be accessed by attendant workers.

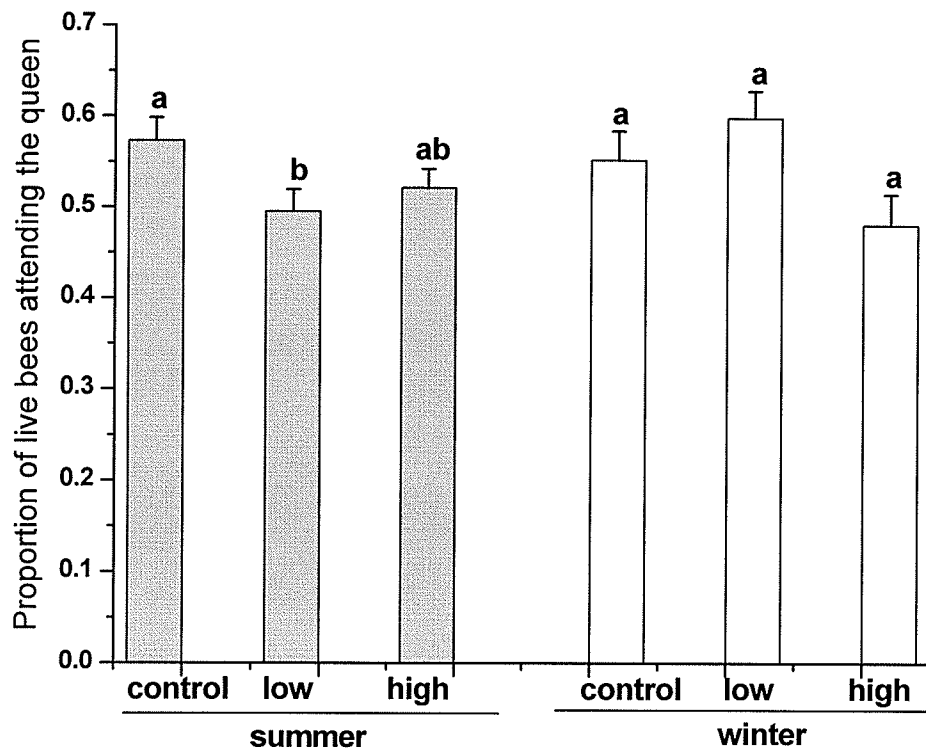


Figure 8.2. The proportion of worker bees (mean \pm SE) attending the queen in a bioassay where bees were fed unadulterated sugar syrup (control) or sugar syrup containing 100 ppm (low) or 1,000 ppm (high) formic acid in summer ($n = 6$) or winter ($n = 2$).

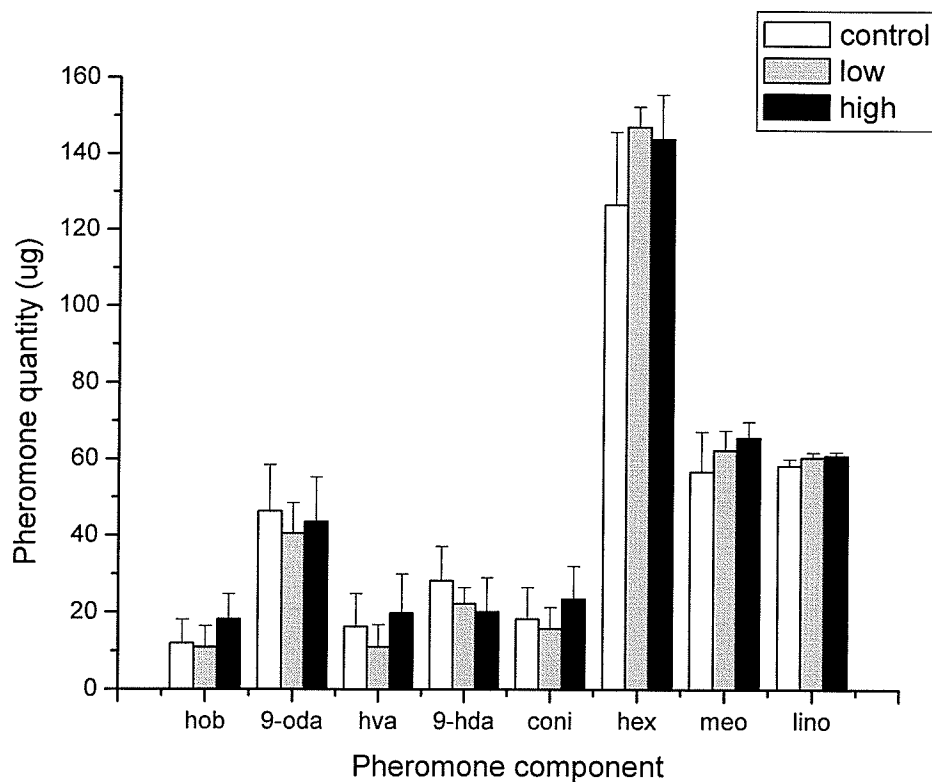


Figure 8.3. Pheromone quantity in honey bee queen body washes (mean \pm SE averaged over the summer and winter trials) from a bioassay where bees were fed unadulterated sugar syrup (control) or sugar syrup containing 100 ppm (low) or 1,000 ppm (high) formic acid ($n = 8$). The quantity of hob changed significantly with season, averaging $18.26 \pm 4.05 \mu\text{g}$ in summer ($n = 6$) and was undetectable in winter ($n = 2$).

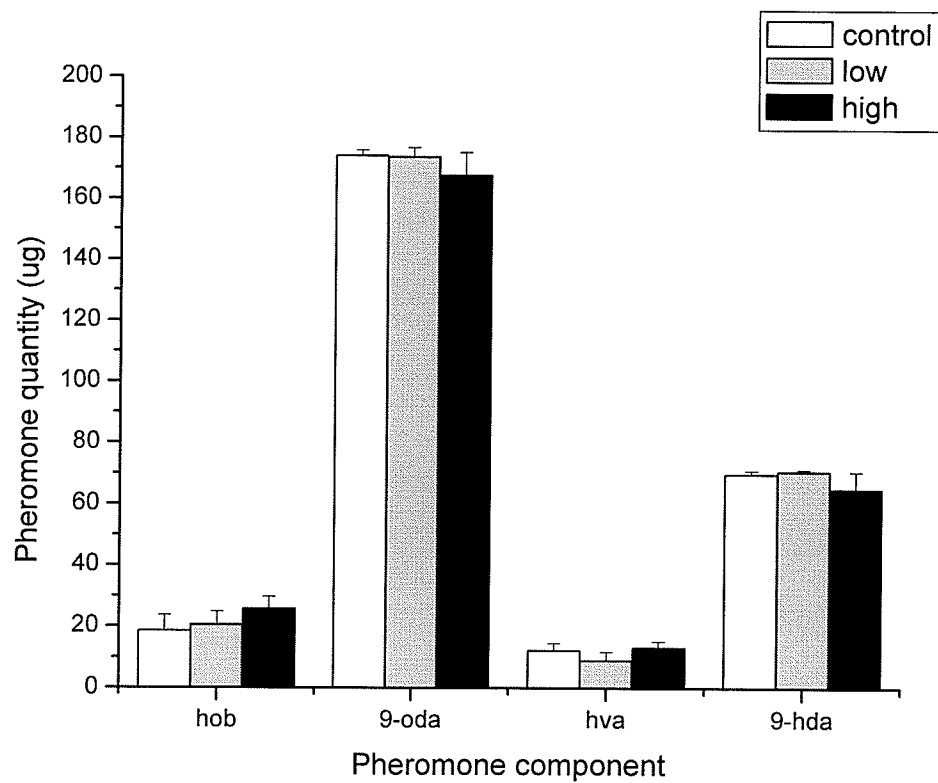


Figure 8.4. Pheromone quantity in honey bee queen mandibular glands from a bioassay where bees were fed unadulterated sugar syrup (control) or sugar syrup containing 100 ppm (low) or 1,000 ppm (high) formic acid ($n = 8$).

GENERAL DISCUSSION

This study examined the use of formic acid as a fumigant indoors in winter. A series of laboratory and field experiments were designed to address the main objectives of 1) understanding how the principles of fumigation as a pest control measure could be applied to formic acid fumigation of honey bees, 2) determining the feasibility of indoor fumigation of honey bee colonies as a pest control technique, and 3) determining the treatment regime that causes the maximum varroa mite mortality while causing the minimum effect on honey bees. I conducted experiments to determine the efficacy of formic acid fumigation against varroa mites, tracheal mites, and nosema spores, the factors affecting formic acid concentration in hives during fumigation, and the effects of fumigation on worker and queen bees.

EFFICACY AGAINST PESTS AND DISEASES

Varroa mites

Outdoor formic acid treatments can effectively control varroa mites. Treating colonies with a pour-on formulation 4 - 6 times at 1 - 4 day intervals kills up to 99 % of varroa mites (Fries, 1989; Hoppe et al., 1989; Mutinelli et al., 1994; Clark, 1994, 1995; Eguaras et al., 1996). However, this treatment method can be expensive in terms of labor costs in commercial operations where there is a need to make additional trips to the apiary to apply multiple treatments and treatment efficacy is highly variable and dependent on climatic conditions (Gatien and Currie, 2003; Bahreini et al., 2004; Ostermann and Currie, 2004; Currie and Gatien, in review). The effectiveness of outdoor slow-release formulations outdoors against varroa mites is also highly variable. Efficacy ranges from 0 to 95 % varroa mite mortality (Clark,

1995; Feldlaufer et al., 1997; Calderone and Nasr, 1999; Calderone, 1999, 2000; Baxter et al., 2000; Eguaras et al., 2001, 2003). The variation in efficacy may be due to the effects of ambient temperature and colony size on the amount of formic acid volatilized into hive air (Feldlaufer et al., 1997; Skinner et al., 2001; Bahreini et al., 2004; Ostermann and Currie, 2004).

The formic acid concentrations that were found to be effective in the laboratory and overwintering building in this study were similar to those measured in formic acid-treated hives during field trials. During slow-release treatments, formic acid levels in hives range from 10 - 64 ppm (Feldlaufer et al., 1997; Skinner et al., 2001; Ostermann and Currie, 2004), while concentrations during pour-on treatments range from 19 - 400 ppm (Feldlaufer et al., 1997; Imdorf et al., 1999; Ostermann and Currie, 2004). Feldlaufer et al. (1997) found that a 21-day fumigation with acid concentrations maintained above 10 ppm kills 61 - 76 % of varroa mites in colonies fumigated outdoors. On the other hand, Ostermann and Currie (2004) did not find a significant reduction in the varroa mite population after a 27-day fumigation with an average concentration of 10 ppm. Other studies where formic acid concentration in hives was measured failed to measure varroa mite mortality (Charriere et al., 1992; Parkman et al., 1999; Skinner et al., 2001). My study is the first to quantify the relationships between formic acid concentration in hive air, exposure time, and treatment efficacy.

In this study, I showed that the efficacy of formic acid against varroa mites was affected by several factors. Formic acid concentration in hive air, temperature, exposure time, and ventilation rate all interacted to affect treatment efficacy. A better understanding of these relationships will allow us to develop more effective systems

for using fumigants to control honey bee pests and diseases in both indoor and outdoor applications.

To determine the effects of formic acid concentration and temperature during fumigation on varroa mite mortality, I conducted a laboratory study to assess the efficiency of formic acid in a controlled environment under a range of temperatures and formic acid concentrations. I showed that the efficacy of treatment with formic acid was significantly affected by temperature. The CT_{50} product of both bees and mites decreased with increasing temperature. Varroa mite mortality was significantly increased at the relatively high concentrations of 40 and 80 ppm of formic acid when applied at 15, 25, or 35 °C, but not at 5 °C. Thus, at low temperatures, a higher concentration of formic acid would be required to kill varroa mites. During indoor wintering in commercial beekeeping operations, temperature is normally held constant at 5 °C. An experiment where room temperature was increased to 10 °C during low-concentration fumigation supported the laboratory results. There was greater efficacy when room temperature was increased to 10 °C than when it was held at 5 °C. Similarly, Bahreini et al. (2004) found that mite mortality was greater in years when temperatures were higher during outdoor fumigation. It is clear that temperature is a factor influencing the efficacy of formic acid treatments. In some cases it may enhance volatilization of the acid resulting in greater in-hive concentrations, but my results show that temperature can also have a direct effect on treatment efficacy.

My results showed that fumigation efficacy was related to the combination of formic acid concentration and exposure time which is commonly expressed as a concentration x time product (CT product) (Bond, 1984). I showed that concentration-response curves need to be tested under different management systems

to develop concrete control recommendations. In the laboratory, higher concentrations killed mites more quickly than lower concentrations. However, the efficiency of formic acid fumigation was lower at higher concentrations than at lower concentrations for most temperature-concentration combinations. This phenomenon is relatively common in insect fumigation studies. Estes (1965) and Harein and Krause (1964) found that for some species of grain beetles, CT_{50} and CT_{95} products increased as the length of exposure to the fumigant increases, indicating decreased fumigation efficiency at longer exposure times. Decreased fumigation efficiency at higher concentrations is often associated with a form of protective stupefaction that results in organisms slowing down their respiration when exposed to a sudden high concentration of a fumigant (Estes, 1965; Bond et al., 1969). In our study, some form of protective stupefaction may have occurred when bees and mites were exposed to high concentrations of formic acid in some treatment combinations, but we do not have any direct evidence of this.

In experiments performed indoors in winter, higher in-hive concentrations were more efficient than lower in-hive concentrations. Using a high concentration of formic acid for 5 days, the in-hive CT_{50} for varroa mites was 49 ppm*days while the CT_{95} was 111 ppm*days. Using a low concentration for 47 or 65 days, the in-hive CT_{50} for varroa mites was 76 ppm*days while the CT_{95} was 728 ppm*days. These CT products are similar to those measured in the laboratory study where CT_{50} products ranged from 14 - 653 ppm*days depending on the concentration and temperature applied.

Although short-term high concentrations based on hive air were more efficient than long-term low concentrations, both required similar amounts of active ingredient to be introduced into the ventilation system to achieve desired in-hive concentrations.

Therefore, in practical terms, there was little difference in cost between the two treatment strategies. Formic acid concentrations as low as 5 ppm in hives killed mites, but required longer fumigation times than higher concentrations. Although some experiments showed treatment efficacy of only 43 %, this was sufficient to bring the mean abundance of varroa mite to below the economic threshold. It is important to note that calculations of treatment efficacy may vary with the initial mean abundance of varroa mites. Acaricide tests conducted on highly infested colonies would likely reduce mite populations to a similar final level as tests conducted on less infested colonies, resulting in higher efficacy values, but similar end results. This may be due, in part, to the limits of sampling small populations of mites and, in part, to the fact that it is difficult to eliminate 100 % of the mite population.

This study showed that indoor winter fumigation with formic acid was effective as a varroa mite control technique. In each experiment using intact honey bee colonies, varroa mite mortality was significantly increased by formic acid fumigation, regardless of the concentration used or duration of fumigation. This increased mite mortality translated into a significant decrease in the mean abundance of varroa mites over the winter in almost every case. Varroa mite mean abundance was not significantly decreased only when the mite infestation was near the limits of detection for this method of sampling (less than two mites per 100 bees) at the start of the experiment or when the duration of fumigation was only two days with low to moderate concentrations of formic acid (CT products of only 4 and 18 ppm*days in treated colonies). Overall, formic acid fumigation indoors in winter was effective at consistently reducing the mean abundance of varroa mites to below spring economic thresholds.

Tracheal mites

Although this study focused on the effects of formic acid on varroa mites and honey bees, the effect of treatments on natural infestations of tracheal mites was also monitored. Formic acid has been successfully used outdoors for the control of tracheal mites (Sharma et al., 1983; Hoppe et al., 1989; Liu, 1991; Wilson et al., 1993; Nelson et al., 1994) where tracheal mite prevalence, or the proportion of bees infested, is significantly reduced using formic acid treatments.

Tracheal mite prevalence was not significantly affected by formic acid fumigation in any of my experiments, but I found that measuring prevalence was not a good sampling method for evaluating efficacy against tracheal mites during indoor wintering. By collecting and dissecting live bees, I showed that tracheal mite mortality was significantly increased by formic acid fumigation. Both low- and high-concentration treatments of formic acid were effective in killing 92 - 99 % of the tracheal mites. Efficacy of outdoor applications ranges from 54 - 100 % (Sharma et al., 1983; Hoppe et al., 1989; Liu, 1991; Wilson et al., 1993; Nelson et al., 1994), but usually requires lower concentrations than are required for effective control of varroa mites (PMRA, 1994). It is clear that concentrations suitable for varroa mite control in our study were also effective in killing tracheal mites. However, the minimum cumulative concentration that would provide effective control of tracheal mites was not determined.

I was also able to demonstrate that environmental conditions can affect tracheal mite populations during winter. DeGuzman et al. (2001) speculated that environmental conditions in different geographic locations may influence tracheal mite survival, but few data are available that clearly establish an environmental effect. In this study, I found that when ventilation rates were manipulated, tracheal mite

survival was affected even in the absence of formic acid. When the ventilation was kept to a minimum, tracheal mite survival was much higher than when ventilation was allowed to increase to control room temperature. The ventilation effect may be due to differences in humidity in the minimum and variable ventilation treatment rooms, but experiments that manipulate humidity independent of other factors would be required to show if this is responsible for the differences in mite mortality over time.

Nosema spores

The effect of treatments on natural infections of nosema disease was also monitored. Acid fumigation with a variety of products has shown mixed results for the suppression of nosema disease. DeRuijter and van der Steen (1989) found that nosema spores on glass plates placed in acetic acid-fumigated hive equipment are inviable and unable to infect healthy bees. However, Fries (1990) fumigated spores with formic acid on wax combs and found that they can still cause infection even after four formic acid applications. In addition, Ostermann (2002) found that formic acid has no effect on nosema disease in outdoor spring applications.

In this study, the change in the mean abundance of nosema spores over winter was significantly affected by formic acid fumigation in only one of four indoor winter fumigation experiments. However, in all cases, nosema spore mean abundance in spring was lower in treated colonies than in untreated colonies. Treated colonies in experiments where no significant effect of formic acid treatment was observed showed, at most, slight increases in nosema spore mean abundance over winter. Thus, formic acid treatment may act to suppress nosema outbreaks, but this study did not provide sufficient support for this hypothesis. This may partly be because our colonies did not develop serious nosema infections. In three of the four experiments, colonies entered the winter with low spore mean abundances and only a small number

of untreated colonies showed large increases over the winter. Further work with colonies inoculated with nosema spores is necessary to determine the efficacy of this treatment against nosema disease. In addition, a laboratory study to determine the formic acid concentration-time combination necessary to control nosema disease would help to clarify if this treatment might be feasible.

Where formic acid treatment suppressed the development of nosema disease relative to untreated controls, a short-term high-concentration treatment was employed. The ability of formic acid fumigation to control nosema disease is likely dependent on the combination of exposure time and concentration applied. It is likely that this combination was not achieved in all of our experiments or in field trials by Ostermann and Currie (2004). Further study is required to determine whether formic acid fumigation could have any benefits in reducing the possibility of winter outbreaks of nosema disease and what treatment regime would be the most efficacious.

FACTORS AFFECTING FORMIC ACID CONCENTRATION

This study is the first reported work with formic acid as a fumigant indoors. A method was established by which formic acid could be distributed throughout experimental rooms. Formic acid concentration in room air was controlled by the use of various solutions of formic acid in water ranging from 25 - 85 % formic acid in conjunction with humidifier plates and sponges that provided relatively consistent concentrations of formic acid in room air. Formic acid concentrations in hive air were also studied as they should relate more directly to varroa mite, worker bee, and queen bee mortality. Quantification of the concentration of formic acid in hive air during fumigation has been attempted by a number of researchers working with formic acid

outdoors (Wissen and Maul, 1981; Feldlaufer et al., 1997; Calderone and Nasr, 1999; Skinner et al., 2001; Ostermann and Currie, 2004). They measured concentrations ranging from 0 - 222 ppm depending on the release method used and method of sampling. These in-hive concentrations were obtained when formic acid was applied directly to the inside of hives where the bees could use ventilation to circulate fresh acid-free air into the hive. In this study, formic acid was applied by introducing acid into the air in rooms containing test colonies. Thus, in this case, ventilation by bees would replace air within the hive with air containing formic acid. Under these conditions, there is still considerable variation in concentration between hives. I identified a number of factors that could affect the acid concentration in hive air during indoor fumigation. These include absorption of acid by honey, the size of the honey bee population, the ventilation rate during fumigation, and the position of the hive in the treatment room.

During indoor winter fumigation, the average concentration of formic acid in the hive air was consistently lower than that in the room air. Even after 65 days, the air in all hives did not equilibrate with the room air. This may be due, in part, to the effect of sorption of acid by honey, hive parts, or the bees themselves. Sorption, which includes adsorption to the surface of a material and absorption into the solid or liquid phase of a solution, is a major factor that can affect the performance of fumigants (Bond, 1984). In general, sorption of a given fumigant tends to be greater at both lower temperatures and higher moisture contents (Bond, 1984). However, the reaction of a particular fumigant cannot be predicted, but must be experimentally tested (Bond, 1984). Work with acetic acid fumigation of seeds showed that acid is readily absorbed by grain during fumigation and this results in substantial reductions in the acid concentration in storage bins (Sholberg and Gaunce, 1996). Similarly,

sorption of formic acid by wooden hive components, wax, honey, or bees may affect the concentration in hives.

Many studies have quantified increases in the natural formic acid content of honey following fumigation (Stoya et al., 1986; Hansen and Guldborg, 1988; Liu et al., 1993; Bogdanov et al., 2002). I showed that both capped and uncapped honey comb filled with honey absorb increasing amounts of formic acid during the first 20 days of acid exposure. The absorption of formic acid by honey alone may be high enough to lower the concentration in hive air. However, differences in the proportion of capped and uncapped honey in the hive may not have a substantial effect on in-hive concentrations, because both absorb formic acid at a similar rate. In addition, hive equipment and the honey bees themselves may affect formic acid concentration in hives. This hypothesis is supported by work by Wissen and Maul (1981) who showed that both wooden hive components and bees have increased formic acid content as a result of fumigation. A controlled experiment is necessary to determine the importance of sorption of acid during fumigation of honey bees.

Wissen and Maul (1981) suggested that bees can affect the formic acid concentration in the hive. In outdoor fumigation, the size of the honey bee population might affect the concentration in the hive by influencing the air flow through the hive. Ostermann and Currie (2004) found that the concentration of formic acid in the hive air during outdoor treatments is negatively correlated with the mean abundance of varroa mites. They suggested that, because colonies without varroa mites have larger honey bee populations than those with mites, volatilization of formic acid from absorbent pads in the hive is increased through increased ventilation.

This study showed that the size of the honey bee population influenced the cumulative concentration of formic acid to which the bees were exposed. The size of

the honey bee population, as measured in spring, was negatively correlated with the CT product in that hive during indoor fumigation. One might expect that, during indoor fumigation, a larger bee population with higher ventilation would circulate more formic acid through the hive resulting in higher in-hive formic acid concentrations. However, other factors correlated with cluster size, such as the quantity of food stored in the hive, may influence in-hive concentrations. Fumigating in early winter when cluster sizes and colony food stores are more uniform may reduce the variability in in-hive formic acid concentrations and, thus, result in more uniformity in the exposure times required to get effective varroa mite control in different colonies. The dynamics of the relationship between bees, ventilation and formic acid concentration in indoor and outdoor applications are poorly understood and require further study.

In addition to standardizing the size of colonies being fumigated, my results suggested other factors that could be managed to increase the uniformity of treatment. The type of ventilation during fumigation influenced in-hive formic acid concentration. When constant (minimum) ventilation was used, variation in formic acid concentration among hives was quite high. However, when variable ventilation was used, the variation in formic acid concentration was diminished. This is likely because when ventilation was increased, formic acid concentration in room air was lowered/or and the room air temperature was more uniform. Further work is required to determine the effect that room ventilation during indoor wintering has on the environment inside the hive. Minor improvements in ventilation and temperature control during indoor winter fumigation may be a feasible method to reduce the variation in concentration between hives and aid in the standardization of the concentration received by fumigated colonies.

My results also showed that the cumulative concentration of formic acid to which the bees were exposed during indoor winter fumigation varied with the position of the hive in the treatment room when hives are stacked more than three high. The acid concentration in hives stacked up to three high was similar. However, colonies above these, in rows 4 and 5, were sometimes exposed to significantly greater cumulative concentrations. This variation did not appear to be due to uneven distribution of vapors in room air. Measurements of the formic acid concentration in room air showed that, when using an air tube ventilation system, formic acid vapors were evenly distributed.

Perhaps the variation in in-hive concentration among hives is due to a factor such as equipment compaction or the type of equipment that is used. Hives lower in the stack may be compressed by hives higher in the stack, making lids fit more tightly. In contrast, the lids on hives on the top of the stack may not be compressed and may, thus, allow for greater air circulation resulting in higher formic acid concentrations. Similarly, the type of equipment that is used may have an effect. For example, hives with no top entrance may have less air circulation than those with a top entrance. In a field study, Wissen and Maul (1981) showed differences in formic acid distribution in hives based on equipment differences. Hives with frames perpendicular to the hive entrance had lower formic acid concentrations than those with frames parallel to the entrance, suggesting that hive equipment is an important consideration during formic acid treatments. In some experiments in this study, equipment type varied because the colonies and hive equipment were donated by a large number of cooperating beekeepers. This may have increased in-hive variability, but would not affect my results because these hives were distributed randomly throughout the treatment. Further work is needed to determine the effect of hive

equipment and stacking on in-hive formic acid concentration during indoor winter fumigation.

The ability to improve the standardization of the concentration of formic acid in hives during fumigation will improve the efficacy of this method on a commercial scale. My results show that this may be achieved through standardization of hive equipment and/or honey bee populations or through improvements in ventilation in wintering buildings. If all hives receive the same cumulative concentration of formic acid, beekeepers should be able to obtain a high degree of efficacy at shorter exposure times.

EFFECTS ON WORKERS, QUEENS, AND QUEEN PERFORMANCE

The potential short- and long-term effects of formic acid fumigation on workers, queens, and intact colonies were examined in a number of experiments. Short-term effects were examined in the laboratory and field by assessing daily worker mortality rates, queen and colony survival, and spring cluster sizes. Long-term effects were examined in the field by assessing brood and honey production and supersedure rates in colonies containing queens that survived winter fumigation.

Worker bees

Adult worker bees exposed to formic acid treatments outdoors can have greater mortality rates than untreated bees (Nelson et al., 1994; Elzen, 2003; Ostermann and Currie, 2004). However, formic acid fumigation does not always negatively affect honey bees. Using a variety of formic acid treatments, researchers have reported no negative effect of formic acid fumigation on bees (Garg et al., 1984; Hoppe et al., 1989; Sharma and Raj, 1999; Skinner et al., 2001; Sharma et al., 1994, 2003; Gatién and Currie, 2003; Currie and Gatién, in review). In this study, worker

bee mortality was increased by formic acid concentration in the laboratory, but significant worker mortality in full-size colonies was observed only when abusive concentration-time combinations were intentionally applied.

In the laboratory, worker bee mortality was significantly increased by formic acid fumigation and the difference in the CT_{50} product for bees and mites was generally quite low. This agrees with Lindberg et al.'s (2001) finding that formic acid is not selective when used in laboratory experiments and that the LD_{50} for worker bees is only twice that for varroa mites. In contrast, when intact honey bee colonies were fumigated with similar concentrations of formic acid, worker bee mortality was generally not increased relative to unfumigated colonies during indoor treatments. While the CT_{50} product was not established for worker bees in full-size colonies in this study because colonies were donated by cooperating beekeepers, it was clearly higher than that measured in my laboratory trial. In the laboratory, CT products as low as 60 ppm*days significantly increased bee mortality. In contrast, full-size colonies under low-concentration indoor fumigation, CT products of over 725 ppm*days in hive air were used without killing significant numbers of workers. Under high-concentration fumigation, significant bee mortality was observed at a CT product of 108 ppm*days in hive air given over only 9 days of indoor fumigation, but no significant worker mortality was observed at slightly lower concentrations when 98 ppm*days were given over 7 days or when 160 ppm*days were given over 10 days. Thus, the combination of formic acid concentration and exposure time is an important factor determining the effect of treatment on bees.

The lack of a direct effect of fumigation on worker bees during indoor winter fumigation indicates that this treatment method can be safely used at a variety of formic acid concentrations and treatment durations. Worker bee mortality was

increased and, subsequently, the spring cluster size was reduced only when an abusive concentration-time combination of formic acid was intentionally applied.

Queen bees

Queen survival was affected by winter formic acid fumigation in some experiments. Queen loss during outdoor formic acid treatment has been reported by some researchers, but was not seen by others. Prior to my research, the causes of formic acid-related queen loss had not been thoroughly examined. No queens were lost in several studies using formic acid treatments outdoors (Bracey and Fischer, 1989; Hoppe et al., 1989; Sharma et al., 1994, 2003; Gatien and Currie, 2003; Ostermann and Currie, 2004; Currie and Gatien, in review) despite the fact that much higher in-hive concentrations of formic acid were used than in my study (Ostermann and Currie, 2004). Queen losses of 5-10 % were reported when rapid-release treatments were used outdoors (VonPosern, 1988; Fries, 1989) and losses of 20 % were reported when slow-release treatments were used outdoors (Wachendörfer et al., 1983; Elzen, 2003). However, the cause of queen loss during formic acid fumigation in these latter studies is not known as the details of the conditions when queen loss occurred were not reported.

Queen loss may be due to a variety of direct or indirect effects of formic acid. High formic acid concentrations may cause increased queen mortality by acute or chronic exposure and/or be affected by the temperature at the time of exposure. On the other hand, formic acid may indirectly cause increased queen mortality when it gets into bee feed or affects the queen's pheromones. In this study, I examined some of the factors that may influence queen mortality during fumigation, such as in-hive formic acid concentration, cumulative exposure, room ventilation rate and formic acid-laden feed.

Queen loss may be related to the concentration of formic acid to which the queen is exposed during fumigation. High-concentration fumigation caused significantly more queen loss than low-concentration fumigation, but only under minimum ventilation conditions. Significant queen loss was seen in experiments where the concentration of formic acid in hives averaged 22 ppm (maximum 30 ppm) for 2 days, 14 ppm (maximum 59 ppm) for 7 days, 12 ppm (maximum 59 ppm) for 9 days, and 16 ppm (maximum 60 ppm) for 10 days. In contrast, no or minimal queen loss was seen when concentrations of formic acid in hives averaged 14 ppm (maximum 25 ppm) for 2 days, 5 ppm (maximum 7 ppm) for 2 days, 3 ppm (maximum 14 ppm) for 5 days, 5 ppm (maximum 18 ppm) for 5 days, 6 ppm (maximum 33 ppm) for 7 days, 6 ppm (maximum 18 ppm) for 27 days, 4 ppm (maximum 28 ppm) for 47 days, and 4 ppm (maximum 24 ppm) for 65 days. Other studies reporting queen loss did not include measurements of in-hive formic acid concentration (Wachendörfer, 1983; VonPosern, 1988; Fries, 1989; Elzen, 2003).

Queen loss was correlated with the maximum concentration of formic acid measured in individual hives with significantly more queens being killed in colonies that had maximum formic acid concentrations over 20 ppm than in colonies with lower maximum concentrations. In contrast, the cumulative concentration (in-hive CT product) to which the queen was exposed was not correlated with the incidence of queen loss. Treatments with very high cumulative concentrations of formic acid in hives (up to 730 ppm*days) did not increase queen mortality when given as low concentrations over long periods of time. This suggests that there may be an upper lethal formic acid concentration for queens. Thus, queen loss is likely due to acute, not chronic exposure to formic acid.

The effect of exposure to high concentrations of formic acid may vary depending on environmental conditions. Studies of formic acid fumigation of full-size colonies outdoors commonly report concentrations in hives around 50 ppm with some reporting concentrations as high as 222 ppm without experiencing queen loss (Feldlaufer et al., 1997; Calderone and Nasr, 1999; Parkman et al., 1999; Skinner et al., 2001; Ostermann and Currie, 2004). A factor such as ambient temperature may be important. My results showed that it may be possible to mitigate the effects of high concentrations of formic acid on queen loss by regulating room temperature and ventilation rates. In this study, the use of step-wise temperature-dependent room ventilation prevented queen loss during high concentration fumigation. This variable ventilation may have prevented the concentrations of formic acid in hives from reaching concentrations greater than 20 ppm during fumigation, which is especially important if short-term high concentration fumigation regimes are used. However, the benefits of using variable ventilation to reduce queen loss may also be related to its effect on room temperature. VonPoseern (1988) was the first to speculate that high ambient temperatures may increase the incidence of queen loss during formic acid fumigation. In this study, queen loss was more likely when room temperatures were allowed to increase above 8 °C during fumigation with high concentrations of formic acid than when temperatures were kept below 6 °C. Whichever is the case, the data show that producers should avoid higher room temperatures if high-concentration fumigation is attempted indoors.

Sublethal effects related to the absorption of formic acid by honey or sugar syrup have not been previously investigated. This study and others have shown that honey absorbs formic acid during fumigation (Stoya et al., 1986; Hansen and Guldberg, 1988; Liu et al., 1993; Bogdanov et al., 2002). In a colony situation, this

syrup would be fed to the queen by her worker attendants (Winston, 1987). While acid-laden syrup was fed at a level that would eventually be lethal to workers, no queens were killed in my cage trials even though they received high formic acid concentrations in their feed. In the laboratory, queens received high concentrations of formic acid in their feed over a much longer period than that which would cause queen loss in colonies that were fumigated with formic acid. Therefore, the absorption of acid by honey and subsequent ingestion by bees is unlikely to be the cause of the queen loss I observed.

It is commonly suggested that formic acid may cause queen mortality by interrupting pheromone production. This does not seem to be the case with short-term treatments in summer where the number of workers attending queens within one hour after treatment is not affected by formic acid (Westcott and Winston, 1999). However, they also did not observe queen loss. During indoor winter treatments, formic acid could cause queen loss by affecting either the queen's ability to produce pheromones, the workers' ability to detect and respond to the pheromone message, or both. In this study, worker bees fed acid-laden sugar syrup in late summer were less attentive to queens when fed 100 ppm formic acid, than when fed 0 or 1,000 ppm formic acid in sugar syrup. This lends some support to the hypothesis that suggests that queen attendance by workers is affected by formic acid treatment. In winter, however, the same concentrations showed no effect on worker attentiveness.

Analysis of the quantity of each component of the queen's pheromone blend showed that pheromone quantity and quality was not affected by the level of formic acid in bee feed. Thus, a disruption in pheromone production caused by formic acid in food fed to the queen is unlikely a major cause of queen mortality. However, we did find a seasonal difference in the queen's pheromone blend and it is possible that

this difference could explain why queens appear to be more susceptible to high concentrations of formic acid in winter than in summer. In this laboratory experiment, queens and workers were kept apart by wire mesh. Thus, the workers were prevented from “balling” the queen. Balling is the term used to describe worker aggression toward and clustering around the queen that results in her death (Huber, 1992 in Yadava and Smith, 1971). Queens might be more susceptible to balling by workers when their pheromone blend is compromised in winter. It is not known whether the lower retinue response I observed could lead to balling in a situation where workers would have full access to queens. The direct effects of formic acid vapors on queen mortality need to be investigated in “summer” and “winter” queens.

Queen performance

For queens that survived winter formic acid fumigation, there was no effect of formic acid treatment on honey production, brood production, or the frequency of supersedure during the subsequent spring and summer. Whether short-term high concentrations or long-term low concentrations of formic acid were used, surviving queens were as vigorous and productive as unfumigated wintered queens. This indicates that there were no long-term effects of winter fumigation on queens. Similarly, Westcott and Winston (1999) and Nelson et al. (1994) measured colony weight gain and population growth in colonies during the summer after spring treatment with formic acid and found that these variables did not vary with treatment supporting the hypothesis that formic acid fumigation has no long-term effects on queen performance.

SUMMARY

This study showed that formic acid fumigation indoors in winter was an effective method of varroa mite control. Treatment could be carried out in such a way as to maximize varroa mite mortality while minimizing worker and queen bee mortality. I was able to demonstrate that in-hive formic acid concentrations could be attained that consistently brought the mean abundance of varroa mites below the economic threshold without killing queens under combinations of room temperature and ventilation that fall within the range used in normal commercial practice. I also showed that concentrations of formic acid that provided good control of varroa mites could provide effective tracheal mite control, but were not consistently effective against nosema disease. Because the ventilation system used in this study was designed to simulate those recommended for commercial wintering buildings (Gruszka, 1998), beekeepers should be able to readily modify their facilities for indoor formic acid fumigation with little additional cost. However, specific recommendations to obtain the correct formic acid concentration will have to be developed for different buildings with different air flow and air exchange rates.

The results showed that either short-term high concentration or long-term low concentration fumigation methods could be used to control varroa mites by indoor winter fumigation with formic acid. These two methods have advantages and disadvantages that must be taken into consideration when applied to commercial wintering buildings. Short-term high concentration fumigation was highly selective, killing varroa mites at a higher rate than bees. Because this method took only a short time to be effective, it would allow producers access to their colonies sooner. This method, however, has a higher risk of queen loss and higher risk of worker bee mortality if high concentration-time combinations are used. Both of these concerns

can be addressed through careful monitoring of formic acid concentrations and management of temperature and ventilation to control in-hive concentrations.

Long-term low concentration fumigation did not increase queen mortality, effectively killed varroa mites and did not increase worker bee mortality, even after 65 days of continuous exposure. The length of time necessary to kill a sufficient proportion of the varroa mite population is the primary disadvantage of this method. In addition, the low concentrations used in this study did not kill as high a proportion of mites as the high concentrations. A slightly higher concentration may prove to be more effective against varroa mites while still maintaining the advantages of this method.

Both the short-term high concentration and the long-term low concentration methods used approximately the same amount of active ingredient and the quantity used was similar to that which would be used in existing outdoor applications. When either of these methods is used, safety precautions must be taken. In this study, a low pressure ambient air breathing apparatus with continuous flow supplied air hood was used to collect varroa mite and bee samples within the rooms during fumigation and staff were trained in its use. This allowed the experimenter to enter the experimental rooms safely during fumigation at the relatively low concentrations of formic acid used in our experiments. Most beekeepers would not likely be equipped or trained with the use of safety equipment to be able to monitor their hives during fumigation. However, because these rooms have relatively high air exchange rates, acid levels in air quickly drop below the time weighted average of 5 ppm of formic acid (safe level for occupational exposure (Thompson, 1992)), so producers could enter the building within days after fumigation was stopped.

Introduction of an alternative varroa mite control technique that is economical and effective is important for commercial honey producers and is especially critical in light of growing concerns about mite resistance to acaricides. Formic acid fumigation of indoor-wintered colonies will save labor costs and may reduce chemical costs, depending on the concentration that is required. Although applicator safety will be a concern, there are some advantages over existing methods. Because all hives can be treated simultaneously, the amount of chemical handling would be greatly decreased as compared to that which is required to treat thousands of individual colonies outdoors using liquid formic acid treatments registered for outdoor use in Canada. Reduced handling would reduce the probability that the applicator will be injured through accidental spills.

This study identified the parameters that must be achieved and avoided during indoor winter fumigation of honey bees with formic acid to provide consistent control of varroa mites with minimum damage to honey bee colonies. The application methods used in this study could be used to achieve appropriate formic acid concentrations in hives in large scale facilities as long as appropriate concentrations in room and hive air are maintained.

THE NEED FOR FUTURE WORK

1. Determine the effect of ventilation and other environmental conditions on varroa mite mortality during indoor wintering in the absence of formic acid.
2. Establish a CT_{50} and CT_{95} product for worker bees fumigated with formic acid in full-size colonies.
3. Establish a CT_{50} and CT_{95} product for queen bees fumigated with formic acid.

4. Clarify the relationship between ambient temperature, formic acid fumigation, and queen mortality during formic acid fumigation.
5. Determine the effect of formic acid vapors on worker bee behavior toward queens.
6. Establish a CT_{50} and CT_{95} product for tracheal mites fumigated with formic acid.
7. Determine the effects of ventilation and other environmental conditions on tracheal mite mortality during indoor wintering in the absence of formic acid.
8. Determine the efficacy of formic acid as a nosema control technique in colonies inoculated with nosema disease in the fall.
9. Determine the effect of absorption of acid by honey, wax, hive equipment, and bees on the concentration of formic acid in hives during indoor winter fumigation.
10. Determine the effect of hive equipment type and lid compression on the concentration of formic acid in hives during indoor winter fumigation.
11. Determine whether cluster size standardization decreases variability in the formic acid concentration among hives during indoor winter fumigation.
12. Determine the time of winter that is most appropriate for successful fumigation.

LITERATURE CITED

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18:265-267.
- Allen, M. D. 1960. The honeybee queen and her attendants. *Animal Behaviour* 8:201-208.
- Allen, O. B. 1983. A guide to the analysis of growth curve data with special reference to SAS. *Computers and Biomedical Research* 16:101-115.
- Amdam, G. V., K. Hartfelder, K. Norberg, A. Hagen, and S. W. Omholt. 2004. Altered physiology in worker honey bees (Hymenoptera: Apidae) infested with the mite *Varroa destructor* (Acari: Varroidae): a factor in colony loss during overwintering? *Journal of Economic Entomology* 97:741-747.
- Anderson, D. L. 2000. Variation in the parasitic bee mite *Varroa jacobsoni* Oud. *Apidologie* 31:281-292.
- Anderson, D. L., and S. Fuchs. 1998. Two genetically distinct populations of *Varroa jacobsoni* with contrasting reproductive abilities on *Apis mellifera*. *Journal of Apicultural Research* 37:69-78.
- Anderson, D. L., and J. W. H. Trueman. 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology* 24:165-189.
- Bahreini, R., G. H. Tahmasebi, J. Nowzari, and M. Talebi. 2004. A study of the efficacy of formic acid in controlling *Varroa destructor* and its correlation with temperature in Iran. *Journal of Apicultural Research* 43:158-161.
- Bailey, L. 1958. The epidemiology of the infestation of the honey bee, *Apis mellifera*, by the mite *Acarapis woodi* Rennie and the mortality of infested bees. *Parasitology* 48:493-506.

- Ball, B. V. 1985. Acute paralysis virus isolates from honeybee colonies infested with *Varroa jacobsoni*. *Journal of Apicultural Research* 24:115-119.
- Ball, B. V., and M. F. Allen. 1988. The prevalence of pathogens in honey bee (*Apis mellifera*) colonies infested with the parasitic mite *Varroa jacobsoni*. *Annals of Applied Biology* 113:237-244.
- Barbier, J., and E. Lederer. 1960. Structure chimique de la substance royale de la reine d'abeille (*Apis mellifera* L.). *Comptes Rendus des Seances de L'Academie de Science Paris* 251:1131-1135.
- Bath, S. S. 1971. Influence of temperature on the effectiveness of paradichlorobenzene fumigation of webbing clothes moth larvae. *Journal of Economic Entomology* 64:989-990.
- Baxter, J. R., M. D. Ellis, and W. T. Wilson. 2000. Field evaluation of Apistan® and five candidate compounds for parasitic control in honey bees. *American Bee Journal* 140:898-900.
- Bogdanov, S., V. Kilchenmann, P. Fluri, U. Bühler, and P. Lavanchy. 1999. Influence of organic acids and components of essential oils on honey taste. *American Bee Journal* 139:61-63.
- Bogdanov, S., J.-D. Charriere, A. Imdorf, V. Kilchenmann, and P. Fluri. 2002. Determination of residues in honey after treatments with formic and oxalic acid under field conditions. *Apidologie* 33:399-409.
- Bolli, H. K., S. Bogdanov, A. Imdorf, and P. Fluri. 1993. Zur Wirkungsweise von Ameisensäure bei *Varroa jacobsoni* Oud und der Honigbiene (*Apis mellifera* L.). *Apidologie* 24:51-57.

- Bond, E. J. 1975. Control of insects with fumigants at low temperatures: response to methyl bromide over the range 25° to -6.7°C. *Journal of Economic Entomology* 68:539-542.
- Bond, E. J. 1984. *Manual of Fumigation for Insect Control*. Food and Agricultural Organization of the United Nations, Plant Production and Protection, Publication No. 54.
- Bond, E. J., J. R. Robinson, and C. T. Buckland. 1969. The toxic action of phosphine: absorption and symptoms of poisoning in insects. *Journal of Stored Products Research* 5:289-298.
- Boot, W. J., J. N. M. Calis, and J. Beetsma. 1992. Differential periods of varroa invasion into worker and drone cells of honey bees. *Experimental and Applied Acarology* 16:295-301.
- Boot, W. J., J. N. M. Calis, and J. Beetsma. 1993. Invasion of *Varroa jacobsoni* into honey bee brood cells: a matter of chance or choice? *Journal of Apicultural Research* 32:167-174.
- Borror, D. J., C. A. Triplehorn, and N. F. Johnson. 1989. *An Introduction to the Study of Insects*. 6th ed. Saunders College Publishing, Toronto, Ontario, Canada.
- Bracey, S., and F. Fischer. 1989. Initial results of the field treatment of honey bee colonies infested with *Varroa jacobsoni* using formic acid in hot climates. *American Bee Journal*. 129:735-737.
- Burgett, M., and I. Burikam. 1985. Number of adult honey bees (Hymenoptera: Apidae) occupying a comb: a standard for estimating colony populations. *Journal of Economic Entomology* 78:1154-1156.

- Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* 83:575-558.
- Butler, C. G. 1960. The significance of queen substance in swarming and supersedure in honey-bee (*Apis mellifera* L.) colonies. *Proceedings of the Royal Entomological Society of London A*. 35:129-132.
- Butler, C. G., and E. M. Fairey. 1964. Pheromones of the honey bee: biological studies of the mandibular gland secretion of the queen. *Journal of Apicultural Research* 3:65-76.
- Calatayud, F., and M. J. Verdu. 1995. Number of adult female mites *Varroa jacobsoni* Oud. on hive debris from honey bee colonies artificially infested to monitor mite population increase (Mesostigmata: Varroidae). *Experimental and Applied Acarology* 19:181-188.
- Calderone, N. W. 1999. Evaluation of formic acid and a thymol-based blend of natural products for the fall control of *Varroa jacobsoni* (Acari: Varroidae) in colonies of *Apis mellifera* (Hymenoptera: Apidae). *Journal of Economic Entomology* 92:253-260.
- Calderone, N. W. 2000. Effective fall treatment of *Varroa jacobsoni* (Acari: Varroidae) with a new formulation of formic acid in colonies of *Apis mellifera* (Hymenoptera: Apidae) in the northeastern United States. *Journal of Economic Entomology* 93:1065-1075.
- Calderone, N. W., and M. E. Nasr. 1999. Evaluation of a formic acid formulation for the fall control of *Varroa jacobsoni* (Acari: Varroidae) in colonies of the honey

- bee *Apis mellifera* (Hymenoptera: Apidae) in a temperate climate. *Journal of Economic Entomology* 92:526-533.
- Calis, J. N. M., W. J. Boot, J. Beetsma, J. H. P. M. VandenEijde, A. deRuijter, and J. J. M. van der Steen. 1998. Control of varroa by combining trapping in honey bee worker brood with formic acid treatment of the capped brood outside the colony: putting knowledge on brood cell invasion into practice. *Journal of Apicultural Research* 37:205-215.
- Callow, R. K., and N. C. Johnston. 1960. The chemical constitution and synthesis of queen substances of the honeybees (*Apis mellifera*). *Bee World* 41:152-153.
- Callow, R. K., J. R. Chapman, and P. N. Paton. 1964. Pheromones of the honey bee: chemical studies of the mandibular secretion of the queen. *Journal of Apicultural Research* 3:77-89.
- Cantwell, G. E. 1970. Standard methods for counting nosema spores. *American Bee Journal* 110:222-223.
- [CCOHS] Canadian Centre for Occupational Health and Safety. 2002. Material Safety Data Sheet: Formic Acid. <http://www.ccohs.ca>. Hamilton, Ontario, Canada. Accessed 12 January 2005.
- Charriere, J. D., A. Imdorf, and V. Kilchenmann. 1992. Ameisensäure-Konzentrationen in der Stockluft von Bienenvölkern. *Schweizerische Bienen-Zeitung* 115:463-469.
- Chen, Y., J. S. Pettis, J. D. Evans, M. Kramer, and M. F. Feldlaufer. 2004. Transmission of Kashmir bee virus by the ectoparasitic mite *Varroa destructor*. *Apidologie* 35:441-448.

- Clark, K. 1994. Control of varroa mites in British Columbia with either formic acid or Apistan. American Bee Journal 134:829.
- Clark, K. 1995. Field trials for recommendations to control varroa mites. Proceedings of the FSAMII Research Symposium, Edmonton, AB, 15 January, Canadian Honey Council, Calgary, Alberta, Canada. Pp. 9-12.
- Cobey, S. 2001. The varroa species complex: identifying *Varroa destructor* and new strategies of control. American Bee Journal 141:194-196.
- Colin, M. E., R. Vandame, P. Jourdan, and S. DiPasquale. 1997. Fluvalinate resistance of *Varroa jacobsoni* Oudemans (Acari: Varroidae) in Mediterranean apiaries of France. Apidologie 28:375-384.
- Collins, A. M., and J. S. Pettis. 2001. Effect of varroa infestation on semen quality. American Bee Journal 141:490-593.
- Crane, E. 1979. Honey: A Comprehensive Survey. Morrison and Gibb Ltd., London, England.
- Crane, E. 1988. Africanized bees, and mites parasitic on bees, in relation to world beekeeping. In Needham, G. R., R. E. Page, Jr., M. Delfinado-Baker, and C. E. Bowman (eds.), Africanized Honey Bees and Bee Mites, Ellis Horwood Limited, Chichester, England. Pp. 1-9.
- Daniels, R. S., A. Hamid, R. E. L. Rogers, and K. MacKenzie. 1999. Membrane-barrier delivery of formic acid, a chemical used for mite control on honey bees (*Apis mellifera*). Journal of Apicultural Research. 38:63-69.
- DeGuzman, L., T. E. Rinderer, J. A. Stelzer, and D. Anderson. 1998. Congruence of RAPD and mitochondrial DNA markers in assessing *Varroa jacobsoni* genotypes. Journal of Apicultural Research 37:49-51.

- DeGuzman, L. I., T. E. Rinderer, V. A. Lancaster, G. T. Delatte, and A. Stelzer. 1999. Varroa in the mating yard: III. The effects of formic acid gel formulation on drone production. *American Bee Journal* 139:304-307.
- DeGuzman, L. I., T. E. Rinderer, G. T. Delatte, J. A. Stelzer, J. L. Williams, L. D. Beaman, V. Kuznetsov, S. J. Bernard, M. Bigalk, and H. Tubbs. 2001. Multi-state field trials of ARS Russian honey bees. 3. Responses to *Acarapis woodi* 1999, 2000. *American Bee Journal* 141:810-812.
- DeJong, D. 1990. Mites: varroa and other parasites of brood. In R. A. Morse and R. Nowogrodski (eds.), *Honey bee pests, predators and diseases*. Cornell University Press, Ithaca, New York, USA. Pp. 200-218.
- DeJong, D., and P. H. DeJong. 1983. Longevity of Africanized honey bees (Hymenoptera: Apidae) infested by *Varroa jacobsoni* (Parasitiformes: Varroidae). *Journal of Economic Entomology* 76:766-768.
- DeJong, D., R. A. Morse, and G. C. Eickwort. 1982. Mite pests of honey bees. *Annual Review of Entomology* 27:229-252.
- Delaplane, K. S., and W. M. Hood. 1997. Effects of delayed acaricide treatment in honey bee colonies parasitized by *Varroa jacobsoni* and a late-season treatment threshold for the southeastern USA. *Journal of Apicultural Research* 36:125-132.
- Delaplane, K. S., and W. M. Hood. 1999. Economic threshold for *Varroa jacobsoni* Oud. in the southeastern USA. *Apidologie* 30:383-395.
- Delfinado, M. D. 1963. Mites of the honeybee in South-East Asia. *Journal of Apicultural Research* 2:113-114.
- Delfinado, M. D., and E. W. Baker. 1974. Varroidae, a new family of mites on honey bees (Mesostigmata: Acarina). *Journal of the Washington Academy of Science*

Delfinado-Baker, M. 1984. *Acarapis woodi* in the United States. American Bee Journal 124:805-806.

Delfinado-Baker, M. 1988. Variability and biotypes of *Varroa jacobsoni* Oudemans. American Bee Journal 128:567-568.

DeLillo, E., A. DiPalma, and G. Nuzzaci. 2001. Morphological adaptations of mite chelicerae to different trophic activities (Acari). Entomologica 35:125-180.

deRuijter, A., and J. J. van der Steen. 1989. Disinfection of combs by means of acetic acid (96%) against nosema. Apidologie 21:503-506.

Donzé, G., and P. M. Guerin. 1994. Behavioral attributes and parental care of varroa mites parasitizing honeybee brood. Behavioral Ecology and Sociobiology 34:305-319.

Donzé, G., and P. M. Guerin. 1997. Time-activity budgets and space structuring by the different life stages of *Varroa jacobsoni* in capped brood of the honey bee, *Apis mellifera*. Journal of Insect Behavior 10:371-393.

Donzé, G., P. Fluri, and A. Imdorf. 1998. A look under the cap: the reproductive behavior of varroa in the capped brood of the honey bee. American Bee Journal 138:528-533.

Drescher, W., and P. Schneider. 1988. The effect of the varroa mite upon the fat body of worker bees and their tolerance of pesticides. In Needham, G. R., R. E. Page, Jr., M. Delfinado-Baker, and C. E. Bowman (eds.), Africanized Honey Bees and Bee Mites, Ellis Horwood Limited, Chichester, England. Pp. 452-456.

- Eguaras, M., S. Quiroga, and O. Garcia. 1996. The control of *Varroa jacobsoni* (Acari: Gamasida) by means of organic acids. *Apiacta* 31:51-54.
- Eguaras, M., M. DelHoyo, M. A. Palacio, S. Ruffinengo, and E. L. A. Bedascarrasbure. 2001. A new product with formic acid for *Varroa jacobsoni* Oud. control in Argentina. I. Efficacy. *Journal of Veterinary Medicine* 48:11-14.
- Eguaras, M., M. A. Palacio, C. Faverin, M. Basualdo, M. L. DelHoyo, G. Velis, and E. Bedascarrasbure. 2003. Efficacy of formic acid gel for varroa control in *Apis mellifera* L.: importance of the dispenser position inside the hive. *Veterinary Parasitology* 111:241-245.
- Eickwort, G. C. 1988. The origins of mites associated with honey bees. In Needham, G. R., R. E. Page, Jr., M. Delfinado-Baker, and C. E. Bowman (eds.), *Africanized Honey Bees and Bee Mites* Ellis Horwood Limited, Chichester, England. Pp. 327-338.
- Eischen, F. A. 1995. Varroa resistance to fluvalinate. *American Bee Journal* 135:815-816.
- Eischen, F. A. 1998. Trials (and tribulations) with formic acid for varroa control. *American Bee Journal* 138:734-735.
- Ellis, M. D., and F. P. Baxendale. 1996. Managing varroa in the midwest. Cooperative Extension Service G96-1302-A, University of Nebraska, Lincoln, Nebraska, USA.
- Elzen, P. J. 2003. Suitability of formic acid to control *Varroa destructor* and safety to *Apis mellifera* in the southwestern U.S. *Southwestern Entomologist* 28:261-266.

- Elzen, P. J., F. A. Eischen, J. B. Baxter, J. Pettis, G. W. Elzen, and W. T. Wilson. 1998. Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations. *American Bee Journal* 138:674-676.
- Estes, P. M. 1965. The effects of time and temperature on methyl bromide fumigation of adults of *Sitophilus granarius* and *Tribolium confusum*. *Journal of Economic Entomology* 58:611-614.
- Feldlaufer, M. F., J. S. Pettis, J. P. Kochansky, and H. Shimanuki. 1997. A gel formulation of formic acid for the control of parasitic mites of honey bees. *American Bee Journal* 137:661-663.
- Floris, I, P. Cabras, V. L. Garau, E. V. Minelli, A. Satta, and J. Troullier. 2001. Persistence and effectiveness of pyrethroids in plastic strips against *Varroa jacobsoni* (Acari: Varroidae) and mite resistance in a Mediterranean area. *Journal of Economic Entomology* 94:806-810.
- Fluri, P., M. Luscher, H. Wille, and L. Gerig. 1982. Changes in weight of the pharyngeal gland and hemolymph titers of juvenile hormone protein and vitellogenin in worker honey bees. *Journal of Insect Physiology* 28:61-68.
- Free, J. B. 1959. The transfer of food between the adult members of a honeybee community. *Bee World* 40:193-201.
- Fries, I. 1989. Short-interval treatments with formic acid for control of *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies in cold climates. *Swedish Journal of Agricultural Research* 19:213-216.
- Fries, I. 1990. Effect from treatment with formic acid for control of *Varroa jacobsoni* on viability of spores of *Nosema apis*. In *Proceedings of the International*

- Symposium on Recent Research on Bee Pathology, Ghent, Belgium, September 5-7 (Apicultural Abstracts 1356/92).
- Fries, I. 1991. Treatment of sealed honey bee brood with formic acid for control of *Varroa jacobsoni*. American Bee Journal 131:313-314.
- Fries, I., A. Aarhus, H. Hansen, and S. Korpela. 1991. Development of early infestations by the mite *Varroa jacobsoni* in honey-bee (*Apis mellifera*) colonies in cold climates. Experimental and Applied Acarology 11:205-214.
- Fries, I., S. Camazine, and J. Sneyd. 1994. Population dynamics of *Varroa jacobsoni*: a model and a review. Bee World 75:5-28.
- Garg, R., O. P. Sharma, and G. S. Dogra. 1984. Formic acid: an effective acaricide against *Tropilaelaps clareae* Delfinado and Baker (Laelaptidae: Acarina) and its effect on the brood and longevity of honey bees. American Bee Journal 124:736-738.
- Gary, N. E. 1961. Queen honey bee attractiveness as related to mandibular gland secretion. Science 133:1479-1480.
- Gatien, P., and R. W. Currie. 1995. Effectiveness of control measures for the varroa mite. Proceedings of the FSAMII Research Symposium, Edmonton, AB, 15 January, Canadian Honey Council, Calgary, Alberta, Canada. Pp. 3-8.
- Gatien, P., and R. W. Currie. 2003. Timing of acaricide treatments for control of low-level populations of *Varroa destructor* (Acari: Varroidae) and implications for colony performance of honey bees. Canadian Entomologist 135:749-763.
- Glinski, Z., and J. Jarosz. 1984. Alterations in haemolymph proteins of drone honey bee larvae parasitized by *Varroa jacobsoni*. Apidologie 15:329-338.

- Gontarski, H. 1951. The pH-relations in the alimentary canal of bee and the influence on nosema development of feeding buffered and unbuffered sugar solutions. XIVth International Beekeeping Congress, Leamington, England.
- Gregorc, A., A. Pogacnik, and I. D. Bowen. 2004. Cell death in honeybee (*Apis mellifera*) larvae treated with oxalic or formic acid. *Apidologie* 35:453-460.
- Gruszka, J. 1998. Beekeeping in Western Canada. Alberta Agriculture, Food and Rural Development, Edmonton, Alberta, Canada.
- Hansen, H., and M. Guldberg. 1988. Residues in honey and wax after treatment of bee colonies with formic acid. *Tidsskrift for Planreavl* 92:7-10.
- Harbo, J. R. 1986. Effect of population size on brood production, worker survival and honey gain in colonies of honeybees. *Journal of Apicultural Research* 25:22-29.
- Harein, P. K., and G. F. Krause. 1964. Dosage-time relationships between 80:20 (CCl₄:CS₂) and adult rice weevils, *Sitophilus oryzae*. *Journal of Economic Entomology* 57:521-522.
- Harris, J. L. 1980. A population model and its application to the study of honey bee colonies. M. Sc. thesis, University of Manitoba, Winnipeg, Manitoba, Canada.
- Herbert, E. W. Jr., P. C. Witherell, W. A. Bruce, and H. Shimanuki. 1989. Evaluation of six methods of detecting varroa mites in beehives, including the experimental use of acaricidal smokes containing Fluvalinate or Amitraz. *American Bee Journal* 128:605-608.
- Hood, W.M., and J. W. McCreadie. 2001. Field tests of the varroa treatment device using formic acid to control *Varroa destructor* and *Acarapis woodi*. *Journal of Agricultural and Urban Entomology* 18:87-96.

- Hoppe, H., W. Ritter, and E. W. C. Stephen. 1989. The control of parasitic bee mites: *Varroa jacobsoni*, *Acarapis woodi* and *Tropilaelaps clareae* with formic acid. American Bee Journal 129:739-742.
- Huang, Z.-Y., and Robinson, G. E. 1995. Seasonal changes in juvenile hormone titers and rates of biosynthesis in honey bees. Journal of Comparative Physiology B 165:18-28.
- Huber, R. 1792. Nouvelles Observations sur les Abeilles. I. English Translation (1926). Dadant and Sons, Inc., Hamilton, Illinois, USA.
- Ifantidis, M. D. 1988. Some aspects of the process of *Varroa jacobsoni* mite entrance into honey bee (*Apis mellifera*) brood cells. Apidologie 19:387-396.
- Imdorf, A., J.-D. Charriere, and P. Rosenkranz. 1999. Varroa control with formic acid. In Proceedings of the Commission of the European Communities Meeting, Agriculture Research Centre – Ghent, Merelbeke, Belgium. 13-14 November. Pp. 24-31.
- Jay, S. C. 1966. Drifting of honeybees in commercial apiaries. III. Effect of apiary layout. Journal of Apicultural Research 5:137-148.
- Jay, S. C. 1968. Factors influencing ovary development of worker honeybees under natural conditions. Canadian Journal of Zoology 46:345-347.
- Jay, S. C., and D. Dixon. 1988. Drifting behaviour and honey production of honeybee colonies maintained on pallets. Journal of Apicultural Research 27:213-218.
- Kaminski, L.-A., K. N. Slessor, M. L. Winston, N. W. Hay, and J. H. Borden. 1990. Honey bee response to queen mandibular pheromone in a laboratory bioassay. Journal of Chemical Ecology 16:841-849.

- Keeling, C. I., K. N. Slessor, H. A. Higo, and M. L. Winston. 2003. New components of the honey bee (*Apis mellifera* L.) queen retinue pheromone. Proceedings of the National Academy of Science 100:4486-4491.
- Klymochko, E. L. 2001. Live dissection: honey bee tracheal mite identification. Manitoba Beekeeper. Fall:12-13.
- Koch, W., and W. Ritter. 1991. Experimental examinations concerning the problem of deformed emerging bees after infestation with *Varroa jacobsoni*. Journal of Veterinary Medicine B 38:337-344.
- Kochansky, J., and H. Shimanuki. 1999. Development of a gel formulation of formic acid for control of parasitic mites of honey bees. Journal of Agricultural and Food Chemistry 47:3850-3853.
- Koeniger, N., and C. Rau. 1980. Feldversuch 1979/1980 mit Ameisensäure im Hochtaunuskreis. Allgemeine Deutsche Imkerzeitung 14:157-159.
- Korpela, S., A. Aarhus, I. Fries, and H. Hansen. 1992. *Varroa jacobsoni* Oud. in cold climates: population growth, winter mortality and influence on the survival of honey bee colonies. Journal of Apicultural Research 31:157-164.
- Kovac, H., and K. Crailsheim. 1988. Lifespan of *Apis mellifera carnica* Pollm. infested by *Varroa jacobsoni* Oud. in relation to season and extent of infestation. Journal of Apicultural Research 27:230-238.
- Krämer, K. 1980. Varroabekämpfung mit Ameisensäure. Die Biene 8:340-343.
- Krämer, K. 1986. Wasserfreie Ameisensäure und die Krämer-Platte. Allgemeine Deutsche Imkerzeitung 20:326-331.
- Kraus, B. 1993. Preferences of *Varroa jacobsoni* for honey bees (*Apis mellifera* L.) of different ages. Journal of Apicultural Research 32:57-64.

- LeConte, Y., and G. Arnold. 1987. Influence de l'age des abeilles (*Apis mellifica* L.) et de la chaleur sur le comportement de *Varroa jacobsoni* Oud. *Apidologie* 18:305-320.
- Lechnitz, K. 1989. Detector tube handbook: air investigations and technical gas analysis with Dräger tubes. 7th ed. Graphische Werkstätten GmbH, Lübeck, Germany.
- Lindberg, C. M., A. P. Melathopoulos, and M. L. Winston. 2000. Laboratory evaluation of miticides to control *Varroa jacobsoni* (Acari: Varroidae), a honey bee (Hymenoptera: Apidae) parasite. *Journal of Economic Entomology* 93:189-198.
- Liu, T. P. 1991. Formic acid and bee mites. *American Bee Journal* 131:311-312.
- Liu, T. P. 1992. Formic acid residues in honey and beeswax. *American Bee Journal* 132:665.
- Liu, T. P. 1996. Varroa mites as carriers of honey-bee chalkbrood. *American Bee Journal* 136:655.
- Liu, T. P., and M. Nasr. 1992. Effects of formic acid treatment on the infestation of tracheal mites, *Acarapis woodi* (Rennie), in the honey bee, *Apis mellifera* L. *American Bee Journal* 132:666-668.
- Liu, T. P., and W. Ritter. 1988. Morphology of some microorganisms associated with the female mite *Varroa jacobsoni*: a survey by electron microscopy. In Needham, G. R., R. E. Page, Jr., M. Delfinado-Baker, and C. E. Bowman (eds.), *Africanized Honey Bees and Bee Mites*, Ellis Horwood Limited, Chichester, England. Pp. 467-474.

- Liu, T. P., L. T. Y. Chu, and P. Sporns. 1993. Formic acid residues in honey in relation to application rate and timing of formic acid for control of tracheal mites, *Acarapis woodi* (Rennie). American Bee Journal 133:719-721.
- Lodesani, M., M. Colombo, and M. Spreafico. 1995. Ineffectiveness of Apistan[®] treatment against the mite *Varroa jacobsoni* Oud in several districts of Lombardy (Italy). Apidologie 26:67-72.
- Lupo, A., and D. Gerling. 1990. A comparison between the efficiency of summer treatments using formic acid and Taktic[®] against *Varroa jacobsoni* in beehives. Apidologie 21:261-267.
- Marcangeli, J. 1997. Natural death rate of *Varroa jacobsoni* (Mesostigmata: Varroidae) in honeybee, *Apis mellifera* (Hymenoptera: Apidae), colonies. Apiacta 32:24-27.
- Martin, S. J. 1994. Ontogenesis of the mite *Varroa jacobsoni* Oud. in worker brood of the honeybee *Apis mellifera* L. under natural conditions. Experimental and Applied Acarology 18:87-100.
- Martin, S. J. 2001. The role of varroa and viral pathogens in the collapse of honeybee colonies: a modelling approach. Journal of Applied Ecology 38:1082-1093.
- Martin, S. J., and A. N. Hogarth. 1994. Varroa research carried out by the National Bee Unit in the United Kingdom. In A. Matheson (ed.), New Perspectives on Varroa, International Bee Research Association, Cardiff, United Kingdom. Pp. 38-40.
- Martin, S. J., and D. Kemp. 1997. Average number of reproductive cycles performed by *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies. Journal of Apicultural Research 36:113-123.

- Martin, S., A. Hogarth, J. vanBreda, and J. Perrett. 1998. A scientific note on *Varroa jacobsoni* Oudemans and the collapse of *Apis mellifera* L. colonies in the United Kingdom. *Apidologie* 29:369-370.
- Maul, V., N. Petersen, and W. Wissen. 1980. Feldversuche zur Varroatosetherapie mit Ameisensäure. *Allgemeine Deutsche Imkerzeitung* 14:155-157.
- Milani, N. 1995. The resistance of *Varroa jacobsoni* Oud to pyrethroids: a laboratory study. *Apidologie* 26:415-429.
- Möbus, S. 1998. Brood rearing in the winter cluster. *American Bee Journal* 138: 511-514.
- Monro, H. A. U. 1969. Manual of Fumigation for Insect Control. Food and Agriculture Organization of the United Nations. Rome, Italy.
- Mozes-Koch, R., Y. Slabezki, H. Efrat, H. Kalev, Y. Kamer, B. A. Yakobson, and A. Dag. 2000. First detection in Israel of fluvalinate resistance in the varroa mite using bioassay and biochemical methods. *Experimental and Applied Acarology* 24:35-43.
- Mutinelli, F., S. Cremasco, and A. Irsara. 1994. Formic acid in the control of varroatosis: a practical approach. *Zentralblatt für Veterinärmedizin* 41:433-440.
- Naumann, K., M. L. Winston, K. N. Slessor, G. D. Prestwich, and F. X. Webster. 1991. The production and transmission of honey bee queen (*Apis mellifera* L.) mandibular gland pheromone. *Behavioural Ecology and Sociobiology* 29:321-332.
- Naumann, K., M. L. Winston, K. N. Slessor, G. D. Prestwich, and B. Latli. 1992. Intra-nest transmission of aromatic honey bee queen mandibular gland pheromone components: movement as a unit. *Canadian Entomologist* 124:917-934.

- Nelson, D. 1995. Population and brood dynamics in indoor wintered colonies. *American Bee Journal* 135:828-829.
- Nelson, D., P. Mills, P. Sporns, S. Ooraikul, and D. Mole. 1994. Formic acid application methods for the control of honey bee tracheal mites. *Bee Science* 3:128-134.
- Oksanen, L. 2001. Logic of experiments in ecology: is pseudoreplication a pseudoissue? *Oikos* 94:27-38.
- Osteen, C. 1993. Pesticide use trends and issues in the United States. In Pimentel, D., and H. Lehman (eds.), *The Pesticide Question: Environment, Economics, and Ethics*, Chapman and Hall, Inc., New York, New York, USA. Pp. 307-336.
- Ostermann, D. J. 2002. Interactions of varroa, *Varroa destructor* Anderson and Trueman, with chalkbrood, *Ascosphaera apis* (Maassen ex Claussen) Olive & Spiltoir, and nosema, *Nosema apis* Zander, in honey bee, *Apis mellifera* L., colonies treated with formic acid and the influence of hive and ambient conditions on formic acid concentration in the hive. M. S. thesis, University of Manitoba, Winnipeg, Manitoba, Canada.
- Ostermann, D. J., and Currie, R. W. 2004. Effect of formic acid formulations on honey bee (Hymenoptera: Apidae) colonies and influence of colony and ambient conditions on formic acid concentration in the hive. *Journal of Economic Entomology* 97:1500-1508.
- Oudemans, A. C. 1904. On a new genus and species of parasitic Acari. Notes From the Leden Museum 24:216-222.

- Pain, J., B. Roger, and J. Theurkauff. 1974. Mise en évidence d'un cycle saisonnier de la teneur en acides céto-9 et décène-2 oïque des têtes de reines vierges d'abeille. *Apidologie* 5:319-355.
- Pankiw, T., M. L. Winston, and K. N. Slessor. 1994. Variation in worker response to honey bee (*Apis mellifera* L.) queen mandibular pheromone (Hymenoptera: Apidae). *Journal of Insect Behavior* 7:1-15.
- Pankiw, T., M. L. Winston, E. Plettner, K. N. Slessor, J. S. Pettis, and O. R. Taylor. 1996. Mandibular gland components of European and Africanized honey bee queens (*Apis mellifera* L.). *Journal of Chemical Ecology* 22:605-615.
- Parkman, J. P., J. A. Skinner, and M. D. Studer. 1999. Comparative efficacy of alternative honey bee mite treatments with an emphasis on formic acid gel activity over time. *American Bee Journal* 139:308.
- Perepelova, L. 1928. [The nurse bees.] *Opytnaya Paseka* 3: 552-557.
- Pettis, J. S. 2001. Biology and life history of tracheal mites. In Webster, T. C., and K. S. Delaplane, *Mites of the Honey Bee*, Dadant and Sons, Inc., Hamilton, Illinois, USA. Pp. 29-41.
- Pettis, J. S. 2004. A scientific note on *Varroa destructor* resistance to coumaphos in the United States. *Apidologie* 35:91-92.
- Pettis, J. S., M. L. Winston, and A. M. Collins. 1995. Suppression of queen rearing in European and Africanized honey bees *Apis mellifera* L. by synthetic queen mandibular gland pheromone. *Insectes Sociaux* 42:1-9.
- Pettis, J. S., H. Shimanuki, and M. F. Feldlaufer. 1998. An assay to detect fluvalinate resistance in varroa mites. *American Bee Journal* 138:538-541.

- Pickard, R. S., and A. A. M. El-Shemy. 1989. Seasonal variation in the infection of honeybee colonies with *Nosema apis* Zander. *Journal of Apicultural Research* 28:93-100.
- [PMRA] Pest Management Regulatory Agency. 1994. Proposed scheduling of 65 percent formic acid for the detection and control of honey bee mites. Submission Management Information Division, Pest Management Regulatory Agency, Govt. of Canada. Note to CAPCO C94-05. March 30, 1994.
- Pugh, P. J. A., P. E. King, and M. R. Fordy. 1992. The respiratory system of the female *Varroa jacobsoni* (Oudemans): its adaptations to a range of environmental conditions. *Experimental and Applied Acarology* 15:123-139.
- Rehm, S.-M., and W. Ritter. 1989. Succession and time of development of male and female offspring of *Varroa jacobsoni* in the worker brood. *Apidologie* 20:508-509.
- Rinderer, T. E., L. I. DeGuzman, V. A. Lancaster, G. T. Delatte, and J. A. Stelzer. 1999. Varroa in the mating yard: I. The effects of *Varroa jacobsoni* and Apistan[®] on drone honey bees. *American Bee Journal* 139:134-139.
- Ritter, W. 1981. Varroa disease of the honeybee *Apis mellifera*. *Bee World* 62:141-153.
- Ritter, W. 1988. *Varroa jacobsoni* in Europe, the tropics, and subtropics. In Needham, G. R., R. E. Page, Jr., M. Delfinado-Baker, and C. E. Bowman (eds.), *Africanized Honey Bees and Bee Mites*, Ellis Horwood Limited, Chichester, England. Pp. 349-359.
- Ritter, W., and F. Ruttner. 1980. Neue Wege in der Behandlung der Varroatose: Ameisensäure. *Allgemeine Deutsche Imkerzeitung* 14:151-155.

- Rogers, L. E., R. O. Gilbert, and M. Burgett. 1983. Sampling honeybee colonies for brood production: a double sampling technique. *Journal of Apicultural Research* 22:232-241.
- Rosenkranz, P., and W. Engels. 1985. Konsequente Drohnenbrutentnahme, eine wirksame biotechnische Maßnahme zur Minderung von Varroatose-Schäden in Bienenvölkern. *Allgemeine Deutsche Imkerzeitung* 19:265-271.
- Ruttner, F. 1983. Varroatosis in honeybees: Extent of infestation and effects. Meeting of EC Experts' Group, Wageningen, 7-9 February. Pp. 7-13.
- Sammataro, D. 1997. Report on parasitic honey bee mites and disease associations. *American Bee Journal* 137:301-302.
- SAS Institute Inc. 1999. SAS/STAT user's guide. SAS Institute, Cary, North Carolina, USA.
- Schatton-Gademayer, K., and W. Engels. 1988. Hemolymph proteins and body weight in newly emerged worker honey bees according to different rates of parasitism by brood mites. *Entomologia Generalis* 14:93-101.
- Schneider, P. 1987. The influence of varroa infestation during pupal development on the flight activity of the worker honey bees. *Apidologie* 18:366-368.
- Schneider, P., and W. Drescher. 1987. The influence of *Varroa jacobsoni* Oud. on weight, development of weight and hypopharyngeal glands, and longevity of *Apis mellifera* L. *Apidologie* 18:101-110.
- Seeley, T. D. 1979. Queen substance dispersal by messenger workers in honey bee colonies. *Behavioral Ecology and Sociobiology* 5:391-415.
- Sharma, S. D., and D. Raj. 1999. Evaluation of different methods of using formic acid against *Acarapis woodi* Rennie infesting *Apis mellifera* L. *Agricultural Science Digest* 19:164-171.

- Sharma, O. P., R. Garg, and G. S. Dogra. 1983. Efficacy of formic acid against *Acarapis woodi* (Rennie). Indian Bee Journal 45:1-2.
- Sharma, S. D., N. P. Kashyup, D. Raj, and A. Kumar. 1994. Effect of formic acid and sulphur on the brood and longevity of adult *Apis mellifera* L. bees. Annals of Biology 10:71-74.
- Sharma, S. D., N. P. Kashyup, and D. Raj. 2003. Efficacy of some acaricides against ectoparasitic mite *Tropilaelaps clareae* infesting European honey bee *Apis mellifera*. Indian Journal of Agricultural Research 37:60-63.
- Sholberg, P. L., and A. P. Gaunce. 1996. Fumigation of high moisture seed with acetic acid to control storage mold. Canadian Journal of Plant Science 76:551-555.
- Simpson, J. 1961. Nest climate regulation in honeybee colonies. Science 133:1327-1333.
- Skinner J.A., J. P. Parkman, and M. D. Studer. 2001. Evaluation of honey bee miticides, including temporal and thermal effects on formic acid gel vapours, in the central south-eastern USA. Journal of Apicultural Research. 40:81-89.
- Slessor, K. N., L.-A. Kaminski, G. G. S. King, J. H. Borden, and M. L. Winston. 1988. The semiochemical basis of the retinue response to queen honey bees. Nature 332:354-356.
- Slessor, K. N., L.-A. Kaminski, G. G. S. King, and M. L. Winston. 1990. Semiochemicals of the honeybee queen mandibular glands. Journal of Chemical Ecology 16:851-860.

- Smith, E. P. 2002. BACI design. *In* El-Shaarawi, A. H. and W. W. Piegorsch (eds.), Encyclopedia of Environmetrics, John Wiley and Sons, Ltd., Chichester, England. Pp. 141-148.
- Snedecor, G. W., and W. G. Cochran. 1980. Statistical Methods. 7th Edition. The Iowa State University Press, Ames, Iowa, USA.
- Spreatico, M., F. R. Eördegh, I. Bernardinelli, and M. Colombo. 2001. First detection of strains of *Varroa destructor* resistant to coumaphos. Results of laboratory tests and field trials. *Apidologie* 32:49-55.
- Stewart-Oaten, A., W. W. Murdoch, and K. R. Parker. 1986. Environmental Impact Assessment: "Pseudoreplication" in time? *Ecology* 67:929-940.
- Stoya, W., G. Wachendörfer, I. Kary, P. Siebentritt, and E. Kaiser. 1986. Ameisensäure als Therapeutikum gegen Varroatose und ihre Auswirkungen auf den Honig. *Deutsche Lebensmittel-Rundschau* 82:217-221.
- Strange, J. P., and W. S. Sheppard. 2001. Optimum timing of miticide applications for control of *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) in Washington State, USA. *Journal of Economic Entomology* 94:1324-1331.
- Strick, H., and G. Madel. 1988. Transmission of the pathogenic bacterium *Hafnia alvei* to honey bees by the ectoparasitic mite *Varroa jacobsoni*. *In* Needham, G. R., R. E. Page, Jr., M. Delfinado-Baker, and C. E. Bowman (eds.), Africanized Honey Bees and Bee Mites, Ellis Horwood Limited, Chichester, England. Pp. 462-466.

- Sylvester, H. A., R. P. Watts, L. I. DeGuzman, J. A. Stelzer, and T. E. Rinderer. 1999. Varroa in the mating yard: II. The effects of varroa and fluvalinate on drone mating competitiveness. *American Bee Journal* 139:225-227.
- Taber, S. 2002. Drifting of bees. *American Bee Journal* 142:813-814.
- Talpay, B. 1989. Inhaltsstoffe des Honigs - Ameisensäure (Formiate). *Deutsche Lebensmittel-Rundschau* 85:143-147.
- Thompson, M. 1992. NTP Technical Report on Toxicity Studies of Formic Acid. United States Department of Health and Human Services. Research Triangle Park, North Carolina, USA.
- Trouiller, J. 1998. Monitoring *Varroa jacobsoni* resistance to pyrethroids in western Europe. *Apidologie* 29:537-546.
- Underwood, R. M., and R. W. Currie. 2003. The effects of temperature and dose of formic acid on treatment efficacy against *Varroa destructor* (Acari: Varroidae), a parasite of *Apis mellifera* (Hymenoptera: Apidae). *Experimental and Applied Acarology* 29:303-313.
- Underwood, R. M., and R. W. Currie. 2004. Indoor winter fumigation of *Apis mellifera* (Hymenoptera: Apidae) colonies infested with *Varroa destructor* (Acari: Varroidae) with formic acid is a potential control alternative in northern climates. *Journal of Economic Entomology* 97:177-186.
- VonPosern, H. 1988. Stopping varroa's victory march part II. *American Bee Journal* 128:425-428.
- Wachendörfer, G., and H. Keding. 1988. Beurteilung von Rückständen im Honig aus der Sicht der amtlichen Lebensmittelüberwachung nach Anwendung von Varroatose-Bekämpfungsmitteln. *Allgemeine Deutsche Imkerzeitung* 22:414-421.

- Wachendörfer, G., E. Kaiser, K. Krämer, and D. Seinsche. 1983. Labor- und Feldversuche mit einem von Krämer modifizierten Ameisensäure-Dämmplatten-Verfahren zur Varroatosebekämpfung. *Allgemeine Deutsche Imkerzeitung* 17:339-344.
- Wachendörfer, G., J. Fijalkowski, E. Kaiser, D. Seinsche, and J. Siebentritt. 1985. Labor- und Feldversuche mit der Illertisser Milbenplatte als neue Anwendungsform der Ameisensäure in Rahmen der Varroatose-Bekämpfung. *Apidologie* 16:291-306.
- Weinberg, K. P., and G. Madel. 1985. The influence of the mite *Varroa jacobsoni* Oud. on the protein concentration and the haemolymph volume of the brood of worker bees and drones of the honey bee *Apis mellifera* L. *Apidologie* 16:421-435.
- Westcott, L. C., and M. L. Winston. 1999. Chemical acaricides in *Apis mellifera* (Hymenoptera: Apidae) colonies; do they cause nonlethal effects? *Canadian Entomologist* 131:363-371.
- Wienands, A., and G. Madel. 1988. Hamozyten der Honigbiene, *Apis mellifera*, und ihre Veränderungen durch Varroatose (Hymenoptera: Apidae). *Entomologia Generalis* 14:81-92.
- Wilson, W. T., J. R. Baxter, A. M. Collins, R. L. Cox, and D. Cardoso-T. 1993. Formic acid fumigation for control of tracheal mites in honey bee colonies. *Bee Science* 3:26-32.
- Winston, M. 1987. *The Biology of the Honey Bee*. Harvard University Press, Cambridge, Massachusetts, USA.

- Winston, M. L., K. N. Slessor, L. G. Willis, K. Naumann, H. A. Higo, M.H. Wyborn, and L.-A. Kaminski. 1989. The influence of queen mandibular pheromones on worker attraction to swarm clusters and inhibition of queen rearing in the honey bee (*Apis mellifera* L.). *Insectes Sociaux* 36:15-27.
- Wissen, W., and V. Maul. 1981. Tests of procedures of application of formic acid in the control of varroa disease. *Apiacta* 61:66-70.
- Wossler, T. C., and R. M. Crewe. 1999. The releaser effects of the tergal gland secretion of queen honeybees. *Journal of Insect Behavior* 12:343-351.
- Yadava, R. P. S., and M. V. Smith. 1971. Aggressive behaviour of *Apis mellifera* L. workers towards introduced queens I. Behavioural mechanisms involved in the release of worker aggression. *Behaviour* 39:211-236.