Final Report

Research Project:

Alternative Methods for Controlling *Varroa destructor* and *Acarapis woodi* Populations in Quebec Hives

Funding Programs:

1. Advancing Canadian Agriculture and Agri-Food (ACAAF) Program
2. Programme d'appui financier aux associations de producteurs désignées – Part 4 "Initiatives" (PAFAPD)

Applicant Organization:

Fédération des apiculteurs du Québec (FAQ)

Collaborating Organizations

Centre de recherche en sciences animales de Deschambault (CRSAD)

Université de Montréal

Université Laval

Foreword

This work was completed between the spring of 2005 and the summer of 2008. It is the result of collaboration and team work. The auteur wishes to thank everyone that participated in the various steps of this project.

Thanks to the funding organizations: The Conseil pour le développement de l'agriculture du Québec (CDAQ) and the Investment Agriculture Foundation of British Columbia through Agriculture and Agri-Food Canada's Advancing Canadian Agriculture and Agri-Food (ACAAF) Program and the Programme d'appui financier aux associations de producteurs désignées – Part 4 "Initiatives" (PAFAPD). Their financial support is vital in acquiring new knowledge in the beekeeping field.

Thanks to the organizations that are coordinating the provincial beekeeping activities and research work: the Fédération des apiculteurs du Québec (FAQ), the Centre de recherche en sciences animales de Deschambault (CRSAD), the Université de Montréal and Université Laval.

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A. Project Description

General Objective

The objective of researches done for this project was to test the various integrated pest management strategies to control *Varroa destructor* and *Acarapis woodi* populations in Quebec hives by avoiding the use of synthetic pesticides. All pesticides tested within this project are organic-based products (formic acid, oxalic acid, thymol, and sucrose powder).

Specific Objectives

Initial Objectives

- To verify whether organic acids applied before, during and after honey flow can be effective in controlling *Varroa destructor* and *Acarapis woodi,* parasites of the honeybee.
- To verify the effectiveness of two commercial products with thymol (Apigard® and Thymovar®) as fall treatments within an integrated pest management strategy against *V. destructor* and *A. woodi*.
- To verify the effectiveness of sucrose powder in reducing the infestation levels of *V. destructor* in honeybee colonies.
- To validate two integrated pest management strategies against varroasis based on intervention thresholds (natural fall of varroa mites in the spring, summer and fall) and to measure the impact of these strategies on acariosis.

Achieved Objectives

All protocols have been completed according to objectives established in the beginning. Work started during 2005 and in the spring of 2007; only thing left to be finalized was the project of validating two integrated pest management strategies. This last protocol was very important, since it targeted the implementation of a combination of control tactics. We completed this protocol

during the 2006-2007 season, and we achieved very satisfying results for one of the two pest management strategies tested. We decided to validate our results by continuing the same protocol with minor adjustments during the 2007-2008 season. Results for this second year confirmed our results and enabled us to suggest an effective integrated pest management method against varroasis.

Use of Hives and Workers

Completing the Part 1 protocols required the use of 604 colonies belonging to two Montérégie beekeepers: Miels Dubreuil (502 colonies) and Miels Gauvin (102 colonies). These beekeepers were responsible for routine operations in apiaries, as well as part of the completion of the different experimental protocols.

The work for Part 2 made use of the hives from the Centre de recherche en sciences animales de Deschambault (CRSAD)'s experimental beekeeping centre and required the use of about a hundred colonies per year since the fall of 2005. The work completed at the CRSAD was carried out by an apiculture technician (Émile Houle), apiary workers (Jean Pierre Lefèvre, Michaël Benoît, and Sylvain Gingras), research assistants (Gabriel Calixte and Georges Martin) and researcher Pierre Giovenazzo.

All these research works were completed under the supervision of the two researchers in charge of the project: Pascal Dubreuil (professor, Université de Montréal's faculty of veterinary science) and Pierre Giovenazzo (contract researcher at the CRSAD).

Research Permit

Tests required the use of organic acaricides, which effectiveness has already been proven by European research. These pesticides are formic acid, oxalic acid, and thymol. Formic acid and oxalic acid are currently certified by the Pest Management Regulatory Agency, Health Canada (PMRA) for use in beekeeping. We contacted the people in charge of the PMRA and they told us that a research permit is not required if these products are used based on federal recommendations. All of our research protocols have used both acids based on the PMRA's health recommendations. In order to test the effectiveness of thymol, we have selected two European commercial products: Apigard® and Thymovar®. Apigard® was used on CRSAD colonies and a research permit from the PMRA was not necessary. However, we asked for the PMRA's permission to use Thymovar® because it was applied on hives belonging to independent

companies (Dubreuil and Gauvin). The protocols were submitted to the PMRA and we received permission of use given the limited number of hives involved.

B. Completed Projects Part 1

1. Effectiveness of thymol in combination with oxalic acid as spring and fall treatment for controlling varroasis and acariosis in Quebec.

Goals

- To measure the effectiveness of thymol (Thymovar®) used alone or in combination with oxalic acid (OA) against varroasis and acariosis in the fall;
- To verify the effects of thymol (Thymovar®) used alone or in combination with OA in the fall and spring on bee colonies during the entire following summer season.

Material and Methods

This project required 240 colonies from Miels Dubreuil. The hives were subdivided into 3 lots of 80 hives, and then they were randomly distributed in the various groups of the experimental protocol (Table 1.1). Treatments A completed in September 2005 are long term treatments. Group 1 colonies were treated with CheckMite® (coumaphos) based on the manufacturer's (Bayer Inc.) recommendations. Group 2 colonies received a treatment with Thymovar® (thymol, one 15 g tab, Vita-Swarm Inc.) in mid-September. Group 3 colonies received a double treatment with Thymovar® (two 15 g tabs): a first treatment in mid-September and a second one in mid-October. Half of colonies treated with Thymovar®, i.e. 40 hives of groups 2 and 3 received a second series of treatments (treatments B) with oxalic acid (dripping of 50 ml of a 1:1 sugar solution at 4% on bees between frames) in mid-November. In the following April, half of all colonies of the different groups 1, 2, and 3 and their subgroups (treatments A) received another series of treatments with oxalic acid in mid-April,

identical to that of the previous fall. In mid-May, half of all the surviving hives of each of the groups and subgroups received a control treatment with CheckMite® (two tabs) for a ten-day period to evaluate the number of varroa mites that survived all treatments since September 2005. The other hives (not controlled with CheckMite® in mid-May) were monitored during the entire summer to evaluate the impact of all treatments until the following fall (2006). In September 2006, these colonies received a final control treatment with CheckMite® (two tabs).

Table 1.1. Experimental protocol and description of experimental treatments. (OA: treatment with oxalic acid by dripping, 50 ml of a 4% solution in a 1 :1 saccharose syrup; WT: without treatment against varroasis at the corresponding date in the table's left column).

Dates	**Experimental Groups**									
Treatments A **Sept 15, 05** **Sept 30, 05**	Group 1 CheckMite®		Group 2 Thymovar®1 One 15 g tab				Group 3 Thymovar® 2 Two 15 g tabs			
Treatments B **Nov 15, 05**	WT		OA		WT		OA		WT	
Treatments C **April 15, 06**	OA	WT	OA	WT	OA	WT	OA	WT	OA	WT
	N=40	N=40	N=20	N=20	N=20	N=20	N=20	N=20	N=20	N=20
May 15, 06	Control treatment with CheckMite® on half of surviving colonies in each subgroup									
Sept 15, 06	Control treatment with CheckMite® on all other surviving colonies									

Screenings of the *Acarapis woodi* mite and the *Nosema sp.* intestinal fungi were completed for each experimental group at the end of the protocol, i.e. on September 3, 2006. For screenings, we took samples of 500 bees from two colonies of each experimental group in each apiary. Bees were put into jars with 70% alcohol and sent to the Laboratoire d'épidémiosurveillance animale du Québec (Saint-Hyacinthe). Evaluations of the levels of parasitism by *A. woodi* and the *Nosema sp.* fungi were completed based on the methodology of the ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) and results were presented in % of parasitized bees in collected samples.

The oxalic and formic acids' residues were measured in honey from a collection of samples of 20 grams of honey and wax per colony taken directly on frames in all colonies of an experimental group. Honey analyses were carried out at the CRSAD with a high-performance liquid chromatography (HPLC) system according to the MAPAQ's methodology.

The combinations of tested treatments are presented in an abbreviated form for presentation purposes. The process being used will combine codes of Table 1 that correspond to the various groups and subgroups. For example, the T1-OA-OA code is indicating the colony group treated with a strip of Thymovar® on September 15, 2005 + oxalic acid on November 15, 2005 + oxalic acid on April 15, 2006.

The dependent variables that are followed are:

- Evolution of varroasis from September 2005 to September 2006: the parasitism level by *V. destructor* is assessed based on the natural fall method. A sticky sheet covering a third of the lower surface is placed under the frames of a colony for a minimum of five days. All these data are presented in daily fall of varroa mites.
- Honey production during the summer of 2006: in kilograms.
- Evolution of the colonies' strength from September 2005 to September 2006: the evaluation of the colonies' strength is given either by the colony's weight (kg) or by the number of frames occupied by bees in the brood chamber (1 to 10).

The statistical analysis of results was completed in two parts. The first series of analyses was completed on results obtained between September 2005 and April 2006 for all 240 experimental colonies at the start. The goal of this first series of analyses is to measure the effect of fall treatments (treatments A and treatments B) on the evolution of varroasis and the evolution of the colonies' strength from the month of September until the colonies are taken out the following spring. The second analysis was completed on results obtained between April 2006 and September 2006 on surviving colonies that were not treated with a CheckMite®

control treatment in May 2006. The goal of this second series of analyses is to measure the impact of the combination of fall and spring treatments on colonies.
These two data groups have been analyzed based on a factorial decomposition of ANOVA to determine the significant effects of treatments (SAS's Proc GLM). The data that did not present a normal distribution (all data on the fall of varroa mites) underwent a rank transformation (SAS's proc rank) to then be analyzed based on the same procedure than for all other data (Proc GLM with ANOVA factorial decomposition).
In the presence of a significant interaction between the different treatments for a variable, a one-way ANOVA (analysis based on groups) was completed; if not, analyses for the different treatments were treated independently. When effects were significant, a LSD (Least Significant Difference α=0.05) contrast test was completed to determine the differences between averages.

Results and Discussion

September 2005 to April 2006

The factorial analysis did not indicate any significant interaction between the primary treatments carried out in the fall of 2005 (C, T1 and T2) and secondary treatments with oxalic acid in the fall and spring. Because of this result, the two treatment groups (Treatments A and Treatments B) are presented in two separate tables. Table 1.2 is presenting the results for primary treatments (Treatments A) that were applied first: the control group with CheckMite®, the experimental group with Thymovar® in a single application and the experimental group with Thymovar® in two applications. Table 1.3 is presenting the results for secondary treatments (Treatments B): presence or absence of an oxalic acid application in the fall in combination with the presence or absence of an identical second treatment the following spring.

Table 1.2. Comparison of the different groups having received the different treatments A based on the protocol. These results come from the first series of analyses obtained between September 2005 and April 2006 for all 240 experimental colonies at the start. The significant differences between groups are indicated by different letters (LSD contrast α<0.05).

Variables	Group 1 CheckMite	Group 2 Thymovar1	Group 3 Thymovar2
Number of active colonies			
- Sept 16, 2005	80 (100%)	80 (100%)	80 (100%)
- Oct 11, 2005	80 (100%)	80 (100%)	80 (100%)
- April 7, 2006	55 (69%)	71 (89%)	66 (83%)
Hive strength (µ±sd, excl. dead bees)			
- Sept 16, 2005 (weight kg)	28.4 ± 0.4 [a]	26.6 ± 0.3 [b]	26.2 ± 0.2 [b]
- Oct 11, 2005 (weight kg)	41.7 ± 0.4 [a]	43.4 ± 0.4 [b]	43.8 ± 0.3 [b]
- Syrup intake Sept 16-Oct 11 (kg)	13.3 ± 0.5 [a]	16.8 ± 0.5 [b]	17.5 ± 0.4 [b]
- Oct 24, 2005 (bee frames)	6.7 ± 0.1 [a]	6.6 ± 0.2 [a]	6.7 ± 0.1 [a]
- April 7, 2006 (bee frames)	6.0 ± 0.2 [a]	5.9 ± 0.2 [a]	5.8 ± 0.2 [a]
- April 8, 2006 (weight kg)	31.0 ± 0.4 [a]	32.2 ± 0.4 [b]	32.4 ± 0.4 [b]
- Intake Oct 11-April 8	11.1 ± 0.4 [a]	12.5 ± 0.7 [a]	11.9 ± 0.4 [a]
Varroasis			
- Sept 11, 2005	0.8 ± 0.14 [a]	0.7 ± 0.1 [a]	0.9 ± 0.12 [a]
- April 13, 2006	0.4 ± 0.01 [a]	0.1 ± 0.05 [a]	0.2 ± 0.07 [a]
- Control w/ coumaphos May 17, 06	0.1 ± 0.05 [a]	2.2 ± 0.79 [b]	1.0 ± 0.58 [b]

Results of the first series of analyses indicate that colony loss between September 2005 and April 2006 is more important in colonies treated with CheckMite®. However, the colonies' strength does not seem to be affected after wintering. All three colony groups come out of wintering with about six bee frames. The difference observed between the weight of colonies of the three groups on April 8 most probably indicates a difference in the nutritional reserves still present in hives and not a difference in the colonies' strength. We noted that syrup intake during the fall was more significant in both groups treated with Thymovar®. This most probably explains the weight difference the following spring. Thymovar® colonies took about 4 kg more syrup than CheckMite® colonies. Despite an impact on syrup intake and the survival of colonies in the winter, the CheckMite® treatment helped reduce varroasis at a significantly lower level the following spring. The control treatment completed in May 2006 decreased varroasis to 0.1 varroa mite/day for CheckMite® as opposed to 2.2 varroa mites/day for the Thymovar®1 treatment and 1.0 varroa mite/day for

the Thymovar®2 treatment. This result also indicates a non-significant Thymovar® dosing effect (α=0.12). In the treatment with two tabs of Thymovar®, varroa mite fall in April is two times lower than the treatment with a Thymovar® tab.

Table 1.3: Comparison of the different treatments B based on the protocol. These results come from the first series of analyses obtained between September 2005 and April 2006 for all 240 experimental colonies at the start. The significant differences between groups are indicated by different letters (LSD contrast α<0.05).

Variables	Without oxalic acid	OA fall
Number of active colonies		
- Sept 16, 2005	160 (100%)	80 (100%)
- Oct 11, 2005	160 (100%)	80 (100%)
- April 7, 2006	123 (77%)	64 (80%)
Hive strength (µ±sd, excluding dead bees)		
- Sept 16, 2005 (weight kg)	27.5 ± 0.2 [a]	26.3 ± 0.3 [a]
- Oct 11, 2005 (weight kg)	42.6 ± 0.3 [a]	43.6 ± 0.4 [a]
- Oct 24, 2005 (bee frames)	6.7 ± 0.1 [a]	6.5 ± 0.2 [a]
- Syrup intake Sept 16-Oct 11 (kg)	15.1 ± 0.4 [a]	17.3 ± 0.4 [a]
- April 7, 2006 (bee frames)	6.3 ± 0.1 [a]	5.2 ± 0.2 [b]
- April 8, 2006 (weight kg)	32.0 ± 0.2 [a]	31.7 ± 0.4 [b]
- Intake Oct 11-April 8	11.2 ± 0.2 [a]	13.2 ± 0.7 [b]
Varroasis		
- Sept 11, 2005 (varroa mites/day)	0.79 ± 0.09 [a]	0.79 ± 0.09 [a]
- April 13, 2006 (varroa mites/day)	0.15 ± 0.04 [a]	0.09 ± 0.02 [b]
- Coumaphos control May 17 (varroa mites/day)	1.13 ± 0.38 [a]	1.00 ± 0.66 [a]

Results of Table 1.3 indicate the impact of secondary treatments with oxalic acid in the fall. Survival is similar with or without treatment with oxalic acid in the fall. However, our work indicates that acid-treated colonies come out of wintering with 1.1 less bee frames than colonies not treated with acid (α<0.05). Oxalic acid-treated colonies consumed 2 kg more during the winter and they are lighter when weighted on April 8, 2006 (α<0.05). Despite this negative impact of oxalic acid on colony wintering, we can see that the varroa mites' fall is significantly lower in April 2006 for acid-treated colonies. This same reduction of varroasis was not measured during the control treatment with CheckMite in May 2006.

April to September 2006

The factorial analysis did not indicate any significant interaction between the primary treatments carried out in the fall of 2005 and secondary treatments with oxalic acid in the winter of 2005 and spring of 2006. Therefore, both treatment groups are presented in two separate tables, like in the previous section. Table 1.4 is presenting the results for primary treatments that were applied first: the control group with CheckMite®, the experimental group with Thymovar®1 and the experimental group with Thymovar®2. Table 1.5 is presenting the results for secondary treatments: presence or absence of an oxalic acid application in the fall in combination with the presence or absence of the application of an identical second treatment the following spring.

Table 1.4. Comparison of the different groups having received the different treatments A based on the protocol. These results come from the second series of analyses obtained between April and September 2006 for surviving colonies in May 2006 and that did not receive the CheckMite® control treatment. The significant differences between groups are indicated by different letters (LSD contrast α<0.05).

Variables	Group 1 CheckMite	Group 2 Thymovar1	Group 3 Thymovar2
Number of active colonies			
- April 7, 2006	12 (100%)	24 (100%)	24 (100%)
- September 22, 2006	11 (92%)	22 (92%)	23 (96%)
Hive strength (µ±sd, excl. dead bees)			
- April 7, 2006 (bee frames)	7.0 ± 0.4 [a]	6.2 ± 0.2 [b]	6.0 ± 0.2 [b]
- Oct 28, 2006 (bee frames)	5.5 ± 0.2 [a]	6.1 ± 0.3 [b]	5.8 ± 0.2 [b]
2006 honey production (kg)			
- Apples (on June 3)	6.6 ± 1.2 [a]	6.0 ± 0.8 [a]	6.3 ± 0.9 [a]
- Blueberries (on June 23)	1.8 ± 1.0 [a]	4.3 ± 0.7 [ab]	5.7 ± 0.9 [b]
- Summer (July 23 to August 15)	14.9 ± 2.7 [a]	19.0 ± 1.8 [a]	21.8 ± 2.2 [a]
- End of summer (August 15 to Sept 13)	11.0 ± 1.6 [a]	11.7 ± 1.0 [a]	12.4 ± 1.0 [a]
- Total	31.2 ± 4.9 [a]	40.0 ± 2.8 [ab]	45.6 ± 2.8 [b]
Varroasis summer 2006 (v. mites/day)			
- April 13	0.05 ± 0.03 [a]	0.20 ± 0.13 [a]	0.12 ± 0.05 [a]
- August 15	0.03 ± 0.02 [a]	0.10 ± 0.02 [a]	0.02 ± 0.01 [a]
- Sept 12	0.15 ± 0.07 [a]	0.26 ± 0.06 [ab]	0.41 ± 0.09 [b]
- Coumaphos control, Sept 18	0.71 ± 0.13 [a]	3.83 ± 0.7 [b]	4.96 ± 0.78 [b]
Acariosis			
September 2006	0.7%	2.0%	0.5%
Nosema disease			
September 2006	Absent	Absent	Absent

The Table 1.4 data indicate the impact of the different primary treatments applied the previous fall (Treatments A) on colonies during the summer of 2006. In the spring of 2006, the CheckMite® colony group was stronger with seven bee frames. This result is not related to the CheckMite® treatment in the fall – it is rather the result of a bias associated with the random selection of colonies completed in May. Despite this experimental bias in favour of the CheckMite® group, we still proceeded with the rest of the analyses.

Results indicate that the three colony groups are presenting an equivalent survival that is above 90% from April to September 2006. Despite the bias in favour of the CheckMite® colonies at the start, this colony group has a

significantly lower strength after the season in October 2006. This strength reduction of colonies to CheckMite® is observed during blueberry pollination, because these colonies produced approximately 3 kg less honey that the two colony groups treated with Thymovar®. The impact of this lower strength of colonies treated with CheckMite® is also observed on the total honey production: the CheckMite® colonies produced 31.2 kg as opposed to 40.1 kg for the Thymovar®1 colonies and 45.6 kg for the Thymovar®2 colonies. Despite this negative impact of CheckMite® as opposed to Thymovar®, we can observe that colonies treated with CheckMite® have significantly lower levels of varroasis in September 2006 (α<0.05). This observation is confirmed by the CheckMite® control treatment completed at the end of the protocol in September 2006. The daily fall is only 0.7 varroa mite/day for the CheckMite® colonies, while it is 3.8 varroa mites/day for the Thymovar®1 colonies and 5.0 varroa mites/day for the Thymovar2® colonies.

Data on the infestation levels by the *A. woodi* parasite are indicating equivalent levels for colonies of the CheckMite® and Thymovar®2 colony groups. The infestation level for the Thymovar®1 colony group is higher, with 2% of infestation of sampled bees. The methodology used to make the evaluation of acariosis allows detecting the presence of this parasite in a colony group and does not provide any precision on the infestation levels per colony. This methodology imprecision drives us to be careful in interpreting these results. Nevertheless, these results are probably indicating a certain level of control by the CheckMite® treatment and the treatment with the application of two Thymovar® tabs (Thymovar®2). The presence of the *Nosema sp.* intestinal fungi has not been detected.

Table 5. Comparison of the different treatments B based on the protocol. These results come from the second series of analyses obtained between April and September 2006. The significant differences between groups are indicated by different letters (LSD contrast α<0.05).

Variables	Without oxalic acid	OA fall	OA spring	OA spring and fall
Number of active colonies				
- April 7, 2006	18 (100%)	12 (100%)	18 (100%)	12 (100%)
- September 22, 2006	17 (94%)	10 (83%)	18 (100%)	11 (92%)
Hive strength (µ±sd, excluding dead bees)				
- April 7, 2006 (bee frames)	6.7 ± 0.3 [a]	5.9 ± 0.2 [bc]	6.6 ± 0.2 [ab]	5.5 ± 0.3 [c]
- Oct 28, 2006 (bee frames)	5.5 ± 0.2 [a]	5.7 ± 0.4 [a]	6.0 ± 0.3 [a]	5.6 ± 0.4 [a]
2006 honey production (kg)				
- Apples (on June 3)	7.2 ± 0.9 [a]	4.8 ± 0.9 [a]	7.1 ± 1.0 [a]	4.8 ± 1.5 [a]
- Blueberries (on June 23)	2.6 ± 1.0 [a]	5.6 ± 1.5 [b]	5.3 ± 0.8 [ab]	4.3 ± 1.0 [ab]
- Summer (July 23 to August 15)	20.2 ± 2.5 [a]	16.2 ± 2.8 [a]	18.2 ± 2.0 [a]	23.2 ± 3.2 [a]
- End summer (Aug. 15 to Sept 13)	11.6 ± 0.9 [a]	12.9 ± 2.1 [a]	11.8 ± 1.2 [a]	11.3 ± 0.8 [a]
- Total	39.2 ± 4.3 [a]	37.4 ± 4.2 [a]	42.3 ± 3.0 [a]	42.6 ± 4.2 [a]
Varroasis summer 06 (v. mites/day)				
- April 13	0.28 ± 0.18 [a]	0.10 ± 0.04 [a]	0.08 ± 0.03 [a]	0.05 ± 0.03 [a]
- August 15	0.07 ± 0.03 [a]	0.00 ± 0.00 [a]	0.04 ± 0.03 [a]	0.02 ± 0.02 [a]
- Sept 12	0.32 ± 0.08 [a]	0.22 ± 0.09 [a]	0.26 ± 0.09 [a]	0.38 ± 0.12 [a]
- Coumaphos control, Sept 18	3.45 ± 0.98 [a]	4.10 ± 0.81 [a]	3.28 ± 0.88 [a]	4.31 ± 1.04 [a]
Acariosis				
September 2006	0.7%	2.0%	0.7%	1.4%
Nosema disease				
September 2006	Absent	Absent	Absent	Absent
Oxalic acid residues (ppm)				
- Apple honey	27.7 ± 1.4	22.0 ± 1.3	27.1 ± 2.1	25.7 ± 1.7

The Table 5 results indicate the impact of the different combinations of treatments with oxalic acid through dripping on colonies between April and October 2006. Globally, the protocol is indicating little impact of oxalic acid on all variables measured as opposed to the control group without treatment with oxalic acid between April and October 2006. The survival levels and the evolution of varroasis within colonies are similar between groups. The only significant difference measured is in blueberry honey production; however, this difference in honey production between groups is disappearing at the end of the season since all four groups have similar honey productions.

The acariosis levels do not help to detect any effect of the treatment with oxalic acid as opposed to the control group. The *Nosema sp.* intestinal fungi is absent in all bee samples.

Analyses of honey samples collected in colonies are not showing any differences between the Table 1.5 groups. Thus, treatments with oxalic acid in the fall and/or spring do not produce any residues in the first honey crop.

Conclusions

- This experimental protocol has helped demonstrate the effectiveness of a fall treatment with Thymovar® (two 15 g tabs) against varroasis.
- The use of Thymovar® has little negative impact on the development of bee colonies when used based on the dosages recommended by European manufacturer Vita-Swarm Inc.
- When oxalic acid is applied at the end of fall, there is a decrease in varroa mites in colonies; however, there is a significant reduction of the colonies' strength of about one bee frame the following spring.
- The treatment with Thymovar® (two 15 g tabs) seems to have some level of control on acariosis.

2. Effect of a decrease in varroa mite populations in the spring on the hive's health and development

Goal

- To verify whether a treatment against varroasis in the days preceding the first honey flow (end of May) allows for a big enough reduction of the parasitic load in colonies to ensure normal development during the summer.

Material and Methods

This project required 42 colonies from Miels Dubreuil and 42 colonies from Miels Gauvin. These colonies were subdivided into three different groups and placed in four different apiaries. The three treatments were applied based on the protocol illustrated in Table 2.1. The three tested treatments are: a control group that did not receive any treatment against varroasis; a group of colonies treated with formic acid (two Mitewipes soaked with 35 ml of 65% formic acid, three applications at five days interval); a colony group treated with oxalic acid (two applications of 5 ml/frame with bees/colony of a 1:1 saccharose solution at 4% of oxalic acid at ten days interval).

Hives were placed inside apiaries in three separate groups based on their experimental group so to minimize bees drifting between groups. Hives have been monitored for the entire summer. A control treatment with coumaphos (two CheckMite® tabs) was made on all colonies in the fall and the varroa mite fall was measured for five days following this treatment.

The oxalic and formic acids' residues were measured in honey from a collection of samples of 20 grams of honey and wax per colony taken directly on frames in all colonies of an experimental group. Honey analyses were carried out at the CRSAD with a high-performance liquid chromatography (HPLC) system according to the MAPAQ's methodology.

The dependent variables that are followed are:

- Evolution of varroasis from April to September 2005: the level of parasitism by *V. destructor* was evaluated based on the natural fall method. A sticky sheet covering a third of the lower surface was placed under the frames of a colony for a minimum of five days. All these data are presented in daily fall of varroa mites.
- Honey production during the summer of 2005: in kilograms.
- Evolution of the colonies' strength from April to October 2005: the evaluation of the colonies' strength is given either by the colony's weight (kg) or by the number of frames occupied by bees in the brood chamber (1 to 10).

Table 2.1. Experimental protocol and description of the various treatments made in the spring.

Group 1	**Group 2**	**Group 3**
Control Without any treatment	Treatment 2 Mitewipes soaked with 35 ml of 65% formic acid 3 applications at 5 days interval	Treatment 5 ml/between frame with bees 1:1 saccharose solution at 4% of oxalic acid 2 applications at 10 days interval
N=6	N=6	N=6

The statistical analyses were completed with the help of the SAS software. For variables that do not respect a normal distribution (the varroa mite fall per day variables), the initial comparison of the three groups was completed through a Kruslkall-Wallis non-parametric analysis with the NPAR1WAY procedure. If the analysis revealed a significant difference ($\alpha<0.05$), a comparison of groups between them was made with a Wilcoxon test. The other variables that respected normal distribution were submitted to a variance analysis with the GLM (generalised linear model) procedure and a LSD (least significant difference) contrast test if need be.

Results and Discussion

Table 2.2 data are indicating all results achieved within the protocol. The colonies' survival level is over 95% in all groups between April and October 2005. Only two colonies are dead on all colonies in the project. The colonies that were not treated in the spring of 2005 (group 1) have a strength in September that is equivalent to the group treated with formic acid. The group treated with oxalic acid is giving the strongest colonies with 8.2 frames and this value is significantly superior to the strength measured in control group 1. The non-treated group also produced significantly less honey than the other two groups during the summer. Natural varroa mite falls measured until September 14 are similar between groups. However, the CheckMite® control treatment has revealed higher infestation levels in the control group and the group treated with oxalic acid. The lowest final varroasis infestation level was measured in the group treated with formic acid with a value of 7.2 varroa mites/day. All three treatments are showing similar formic and oxalic acid residue levels in honey harvested during the summer.

Table 2.2. Comparison of the groups having received the different spring treatments based on the protocol. The significant differences between groups are indicated by different letters (α<0.05).

Variables	Group 1. Control Without treatment	Group 2. Treatment Formic acid	Group 3. Treatment Oxalic acid
Number of active colonies			
- April 13	28 (100%)	28 (100%)	28 (100%)
- May 18	28 (100%)	28 (100%)	28 (100%)
- July 1	28 (100%)	28 (100%)	28 (100%)
- September 14	27 (96%)	28 (100%)	27 (96%)
Hive strength (excluding dead bees)			
- April 13 (bee frames, µ±sd)	6.6 ± 0.2 [a]	6.6 ± 0.2 [a]	6.6 ± 0.2 [a]
- June 8 (bee frames, µ±sd)	11.4 ± 0.6 [a]	11.1 ± 0.6 [a]	11.9 ± 0.5 [a]
- September 14 (bee frames, µ±sd)	7.3 ± 0.4 [a]	8.1 ± 0.3 [ab]	8.2 ± 0.4 [b]
2006 honey production			
- Total June to September	22.8 ± 1.6 [a]	27.9 ± 1.5 [b]	27.5 ± 1.7 [ab]
Varroasis summer 2006 (varroa mites/day)			
- May 13; pretreatment	0.2 ± 0.1 [a]	0.2 ± 0.1 [a]	0.2 ± 0.1 [a]
- May 18; during treatment	0.2 ± 0.1 [a]	0.2 ± 0.1 [a]	1.2 ± 1.0 [a]
- July 1	0.1 ± 0.1 [a]	0.1 ± 0.03 [a]	0.1 ± 0.1 [a]
- September 14; pretreatment	1.4 ± 1.2 [a]	0.3 ± 0.2 [a]	1.3 ± 1.0 [a]
- September 14 to 19, coumaphos control	24.3 ± 12.9 [a]	7.2 ± 2.1 [a]	59.1 ± 40.7 [a]
Acid residues in honey (ppm, µ± sd)			
Oxalic acid (N=6 honey samples)	17.1 ± 2.4	16.4 ± 1.9 (N=6)	16.4 ± 1.9 (N=6)
Formic acid (N=6 honey samples)	0	0	0

Conclusions

- Spring treatments with formic acid (2 Mitewipes soaked with 35 ml of 65% formic acid, 3 applications at 5 days interval) are more effective that treatments with oxalic acid (2 applications of 5 ml/frame with bees/colony of a 1:1 saccharose solution at 4% of oxalic acid at 10 days interval) to reduce the development of varroasis during the summer.
- The absence of treatment in the spring (oxalic or formic acid) is giving weaker colonies in September.
- Spring treatments with oxalic and formic acids are not producing detectable residues in honey taken directly in colonies during the first honey flow.

3. Effect of a decrease in varroa mite populations in August on the hive's health and development

Goals

- To verify whether the effectiveness of anti-varroa mite treatments with oxalic and formic acids in August is enough to let the hive reach, without danger, the mid-September period where a treatment outside honey flow can be applied;
- To measure the levels of acids in honeys harvested in the fall;
- To verify whether hives are behaving normally after these treatments.

Material and Methods

This project required 60 colonies from Miels Dubreuil and 40 colonies from Miels Gauvin. The experimental protocol is consisting of a control group and three experimental groups that received treatments against varroasis during the August honey flow (Table 3.1). The control group colonies did not received any treatment; group 2 colonies received a 65% formic acid treatment with the use of pads soaked with formic acid (35 ml Mitewipe, 2 applications at 14 days interval between the 2 honey supers); group 3 colonies received a 65% formic acid treatment applied in liquid form directly on the hives' tray ("Flash" or ad hoc method, two 35 ml applications at 14 days interval); group 4 colonies received an oxalic acid treatment in a sugar solution poured directly on bees between the frames of the brood super (dripping method, 4% oxalic acid in a saccharose solution, 10 ml per frame space with bees, 2 applications at 7 days interval). The 100 hives were divided into 6 different apiaries, each apiary receiving an equal number of colonies from the different experimental groups.

Table 3.1. Experimental protocol and description of treatments made in the different groups during the month of August.

Group 1	Group 2	Group 3	Group 4
Control Without any treatment	Treatment 2 Mitewipes soaked with 35 ml of 65% formic acid Placed between honey supers 2 applications at 14 days interval	Treatment Application of 35 ml of liquid 65% formic acid Directly on the hive's lower tray 2 applications at 14 days interval	Treatment 1:1 saccharose solution at 4% of oxalic acid Dripping of 10 ml/between frame with bees in the brood super 2 applications at 7 days interval
N=15 (Dubreuil) N=10 (Gauvin)	N=15 (Dubreuil) N=10 (Gauvin)	N=15 (Dubreuil) N=10 (Gauvin)	N=15 (Dubreuil) N=10 (Gauvin)

The colonies' selection and preparation started at the end of July once all of the summer honey had been harvested/taken out. The first series of treatments started on August 15, 2005 and the second series of treatments started on August 30, 2005. Treatments were carried on even if there was a honey flow under way. During the second week of September, all colonies received a CheckMite® (coumaphos) control treatment applied according to the manufacturer's instructions (two tabs placed in the brood super for 42 days).

A screening of *Acarapis woodi* and *Nosema sp.* was completed in each experimental group on September 3, 2006. For this screening, we took samples of 500 bees from two colonies of each experimental group in each apiary. Bees were put into jars with 70% ethanol and sent to the Laboratoire d'épidémiosurveillance animale du Québec (Saint-Hyacinthe). The levels of parasitism by the *A. woodi* mite and the *Nosema sp.* fungi were determined based on the MAPAQ's methodology and results are presented in % of parasitized bees in collected samples.

The oxalic and formic acids' residues were measured in honey from a collection of samples of 20 grams of honey and wax per colony taken directly on frames in all colonies of an experimental group. Honey analyses were carried out at the

CRSAD with a high-performance liquid chromatography (HPLC) system according to the MAPAQ's methodology.

The dependent variables measured are the following:

- Evolution of varroasis from August to September 2005: the level of parasitism by *Varroa destructor* is evaluated based on the natural fall method. A sticky sheet covering a third of the lower surface was placed under the frames of a colony for a minimum of five days. All these data are presented in daily fall of varroa mites.
- Honey production in August and September 2005: in kilograms.
- Evolution of the colonies' strength from August to October 2005: at the beginning of the protocol (August), the colonies' strength was evaluated by using a relative scale varying from 1 (weakest) to 3 (strongest); afterwards, strength was measured by the number of frames occupied by bees in the brood chamber (1 to 10).

The statistical analyses were completed with the help of the SAS software. For variables that do not respect a normal distribution (the varroa mite fall per day variables), the initial comparison of the four groups was completed through a Kruslkall-Wallis non-parametric analysis with the NPAR1WAY procedure. If the analysis revealed a significant difference (α<0.05), a comparison of groups between them was made with a Wilcoxon test. The other variables that respected normal distribution were submitted to a variance analysis with the GLM (generalised linear model) procedure and a LSD (least significant difference) contrast test if need be.

Results and Discussion

Table 3.2 is indicating results achieved. On September 26, there is a lower colony survival level (72%) in the colony group having received the treatment with formic acid applied through the ad hoc method (group 3, "Flash") as

opposed to the other three groups. However, colony loss is similar between the September 2005 and April 2006 groups. There is not any significant difference in the strength between groups in October, but the group 3 colonies ("Flash") are weaker after the wintering period with 6.1 bee frames/brood. This value is significantly inferior to that measured for group 2 colonies (7.9 bee frames, formic acid-Mitewipe), but similar to those of the other two groups. End of summer honey production is revealing similar results, with significantly lower values for the group 3 and 4 colonies, while colonies without treatment (group 1) produced significantly more honey with 11.1 kg.

The natural varroa mite fall is similar between August and September 2005. However, the CheckMite® control treatment made in September 2005 is showing important differences between all four groups. Colonies without treatment (group 1) had a varroa mite fall that was superior than for the three other groups, while colonies treated with formic acid through the ad hoc method (group 3) had the lowest fall.

The honey analyses from colonies of the different experimental groups indicate that formic acid was incorporated into the harvested honey. We measured an average concentration of formic acid in honey of 401 ppm in the group 2 colonies (Mitewipe method) and 228 ppm in the group 3 colonies (ad hoc method).

The acariosis levels are lower in both groups treated with formic acid and the control group. We measured a 5.5% level in colonies treated with formic acid.

Table 3.2. Comparison of the groups having received the different summer treatments based on the protocol. The significant differences between groups are indicated by different letters (α<0.05).

Variables	Group 1 Without treatment	Group 2 Formic acid Mitewipe	Group 3 Formic acid Flash	Group 4 Oxalic acid dripping
Number of active colonies				
- August 15, 2005	25 (100%)	25 (100%)	25 (100%)	25 (100%)
- September 26, 2005	25 (100%)	23 (92%)	18 (72%)	23 (92%)
- October 28, 2005	24 (96%)	23 (92%)	18 (72%))	23 (92%)
- April 7, 2006	22 (88%)	23 (92%)	16 (64%)	20 (80%)
Hive strength (µ±sd, excl. dead bees)				
- Aug. 2, 2005 (relative score:1-2-3)	2.1 ± 0.1 [a]	2.1 ± 0.1 [a]	2.1 ± 0.1 [a]	2.0 ± 0.1 [a]
- October 28, 2005 (bee frames)	6.5 ± 0.4 [a]	6.6 ± 0.4 [a]	6.9 ± 0.4 [a]	7.3 ± 0.3 [a]
- April 7, 2006 (bee frames)	7.2 ± 0.5 [ab]	7.9 ± 0.3 [a]	6.1 ± 0.7 [b]	7.1 ± 0.5 [ab]
2005 honey production				
- Total August/September	11.1 ± 1.4 [a]	9.6 ± 1.2 [ab]	7.4 ± 1.2 [b]	7.1 ± 1.2 [b]
Varroasis summer 2005 (v. mites/day)				
- August 15; pretreatment	0.5 ± 0.1 [a]	0.4 ± 0.2 [a]	0.4 ± 0.1 [a]	0.3 ± 0.1 [a]
- August 30;	0.7 ± 0.3 [a]	0.8 ± 0.4 [a]	0.3 ± 0.1 [a]	0.3 ± 0.1 [a]
- September 12	1.5 ± 0.8 [a]	2.5 ± 1.3 [a]	1.1 ± 0.5 [a]	0.7 ± 0.3 [a]
- Sept 21 to 26, coumaphos control	29.3 ± 9.0 [a]	24.1 ± 11.4 [ab]	5.2 ± 1.2 [b]	14.3 ± 4.7 [ab]
Acariosis				
September 2005	0.5%	1.3%	0%	5,5%
Nosema disease				
September 2005	Absent	Absent	Absent	Absent
Acid residues in honey (µ±sd)				
Oxalic acid (ppm)	37.2 ± 3.0	38.2 ± 6.7	67.4 ± 13.4	54.9 ± 6.8
Formic acid (ppm)	42.4 ± 9.2	401.0 ± 41.2	228.0 ± 4.1	43.1 ± 1.1

Conclusions

- Treatments against varroasis in August with oxalic acid and formic acid as applied in this protocol have reduced the varroasis levels in colonies. The most important reduction was with the treatment of ad hoc application of liquid formic acid on the colonies' lower tray and the dripping of an oxalic acid solution;
- The ad hoc application of liquid formic acid on the colonies' lower tray caused a colony loss, a reduction of the surviving colonies' strength, and a decrease in honey production;
- Following the tested formic acid treatments, residues from this acid are found in the harvested honey.

- Samples of *A. woodi* in experimental colonies seem to show a certain level of control of this parasite through formic acid treatments.

4. Searching risk factors than could be associated with wintering mortality

Goal

- To verify the effect of parasitism, location and August harvest on wintering performances.

Introduction

For the last few years, considerable hive losses have been noted with several beekeepers during wintering. These losses of 25-35% have often been linked to the presence of the *Varroa destructor* parasite. However, in the last two seasons, an adequate control of the level of infestation by this parasite was completed with a majority of Montérégie and Quebec commercial beekeepers and despite this, the wintering mortality level remained at around 25%, considerably above the normal levels of 5 and 10%.

These mortalities happen in the winter and/or spring. Bees seem to die very suddenly (bees remain in a group and often die in cells) or in the spring after being taken out of wintering when excessive and continuous mortality is happening until the complete annihilation of the colony.

This early bee mortality during wintering or post-wintering has been happening for a few years and has often been associated with the presence of varroa mites. In fact, despite adequate control of varroa mites, some colonies are greatly affected by this syndrome, while others are not affected at all. Preliminary results indicate that these rapidly-fading hives are not affected by acariosis or nosema disease; however, last year, viral research helped detect the presence of the deformed wing virus and the black queen cell virus, but without drawing specific conclusions regarding their presence. We are therefore formulating the following assumption: mortalities observed during wintering and in the spring could be

associated with the presence of the *Varroa destructor* parasite or the *Acarapis Woodi* parasite, or with the preparation of the hive in August (activity, harvest, region).

Material and Method

This project required 170 colonies from Miels Dubreuil (2 apiaries of 25 colonies and 6 apiaries of 20 colonies). This protocol's colonies were distributed in various locations of the Montérégie region (Figure 4.1); they were normally managed and their health state and production was monitored. We monitored colonies in the different locations according to a beekeeping cycle, starting with the beginning of pollination activities until the end of wintering. All colonies received an anti-varroa mite treatment with Thymovar® on September 12, 2005 (one strip), followed by a treatment with oxalic acid through dripping (4% in a 1:1 saccharose solution, 5 ml per frame space with bees) on October 24, 2005.

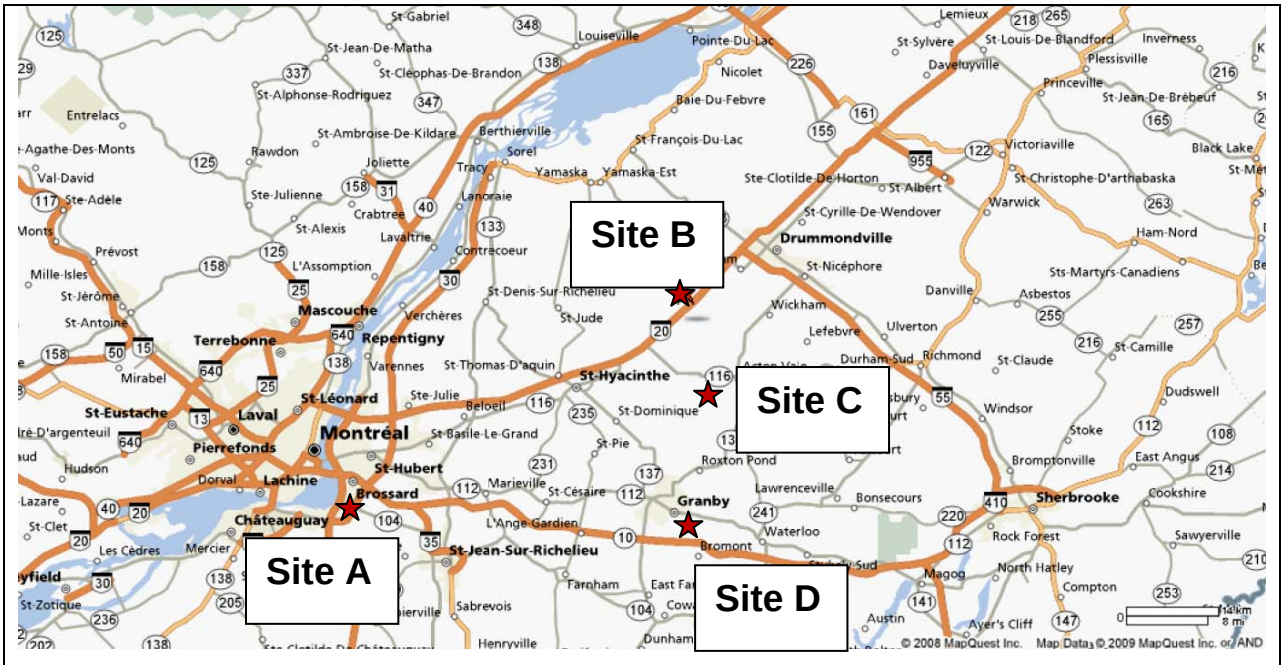


Figure 4.1. Location of the different sites in the Montérégie region.

Description of sites and colonies:

- Site/Group A: Montreal region, two locations in a semi-rural zone located on the south shore, just south of the Champlain Bridge. These mother colonies were placed on these locations following two movements for apple pollination (Montérégie) and blueberry pollination (Lac-Saint-Jean).
- Site/Group B: Saint-Eugène region, two locations in a rural zone. These mother colonies were placed on these locations following four movements for apple (Montérégie), blueberry (Lac-Saint-Jean), cranberry (Centre-du-Québec), and cucumber (Montérégie) pollination.
- Site/Group C: Upton region, two locations in a rural region. These colonies come from nuclei installed in June during blueberry pollination. These colonies were placed on these locations following three movements for apple (Montérégie), blueberry (Lac-Saint-Jean), and cranberry (Centre-du-Québec) pollination.
- Site/Group D: Granby region, two locations in a rural region. These mother colonies were placed on these locations following two movements for apple pollination (Montérégie) and blueberry pollination (Lac-Saint-Jean).

A screening of *Acarapis woodi* and *Nosema sp.* was completed in each experimental group on September 3, 2006. For this screening, we took samples of 500 bees from two colonies of each experimental group in each apiary. Bees were put into jars with 70% alcohol and sent to the Laboratoire d'épidémiosurveillance animale du Québec (Saint-Hyacinthe). Determining the level of parasitism by the *Acarapis woodi* mite was completed based on the MAPAQ's methodology and results are presented in % of parasitized bees in collected samples.

The intra-colony temperature's evolution was monitored with thermo sensors placed directly on the brood super of a colony picked at random. Temperature was taken every hour. In the month of July, sensors were placed in a thermal stability location (work desk) to verify their accuracy. Starting on August 1, all sensors were placed in colonies selected from each location until September 24.

The dependent variables measured are the following:

- Evolution of varroasis from in September 2005: the level of parasitism by *Varroa destructor* is evaluated based on the natural fall method. A sticky sheet covering a third of the lower surface was placed under the frames of a colony for a minimum of five days. These data are presented in daily fall of varroa mites.
- Honey production specific to the crop and total production in 2005: in kilograms.
- Evolution of the colonies' strength from July 2005 to May 2006: at the beginning of the protocol (July), the colonies' strength was evaluated by using a relative scale varying from 1 (weakest) to 3 (strongest); afterwards, strength was measured by the number of frames occupied by bees in the brood chamber (1 to 10).

The statistical analyses were completed with the help of the SAS software. For variables that do not respect a normal distribution (the varroa mite fall per day variables), the initial comparison of the four groups was completed through a Kruslkall-Wallis non-parametric analysis with the NPAR1WAY procedure. If the analysis revealed a significant difference (α<0.05), a comparison of groups between them was made with a Wilcoxon test. The other variables that respected normal distribution were submitted to a variance analysis with the GLM (generalised linear model) procedure and a LSD (least significant difference) contrast test if need be.

Results and Discussion

Figures 4.2 are showing the evolution of the intra-hive temperature in a control colony that is randomly selected in each location of the study. Sensors were placed in colonies on August 1 following a preliminary period in a thermal stability location (work desk). Results indicate that the thermal differences in colonies are significant. They happen every day based on the day/night cycle and also between days. We measured more important temperature differences in the Upton location colony, and less important temperature differences in the Granby colony.

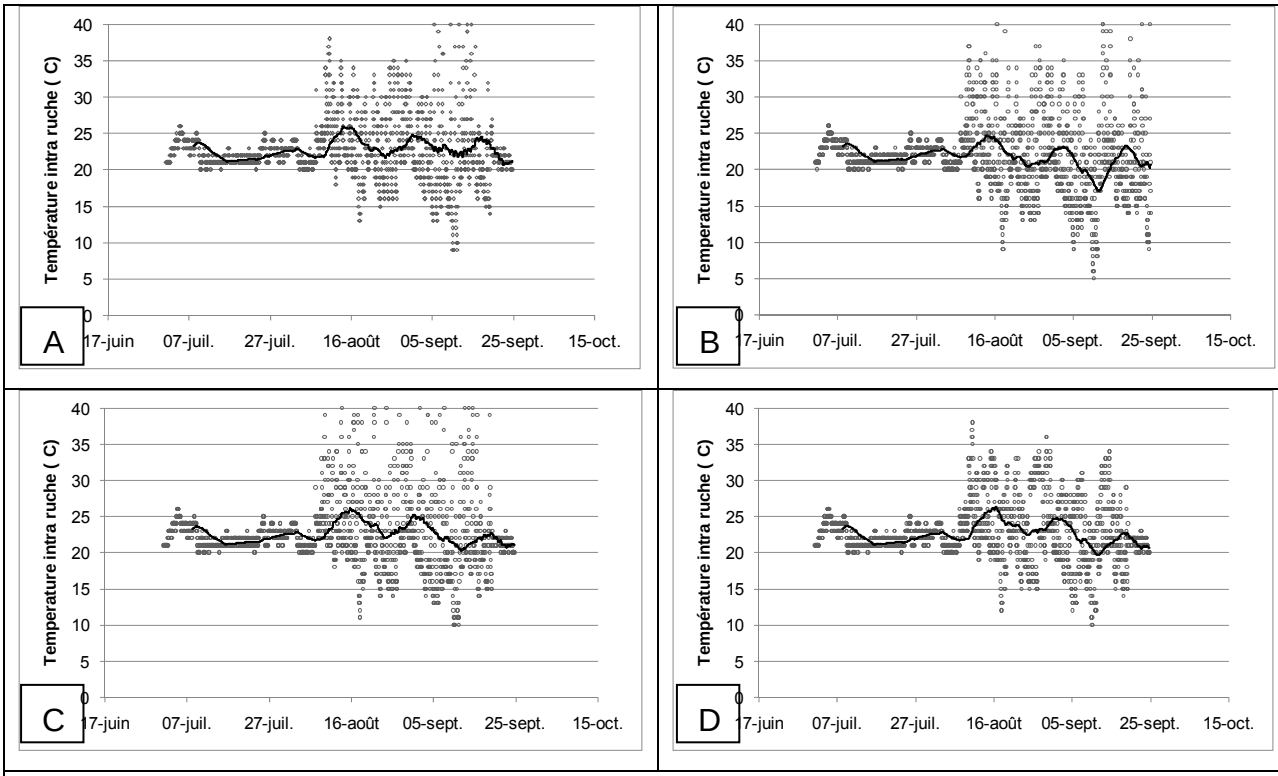


Figure 4.2. Overview of the intra-hive temperature's evolution in the locations of each region under study. The thermo sensor was placed in the colony starting on August 1. The solid line represents the evolution of the hourly temperature's moving average on a 7-day period. A. Montreal region; B. Saint-Eugène region; C. Upton region; D. Granby region.

Table 4.1 is indicating results achieved. The survival levels are above 90% in all locations in September 2005. However, after wintering in April 2006, there was

lower survival for colonies in the Montreal region (80%). The intra-colony monthly hourly average temperatures are not indicating significant differences between all four locations. However, the temperature of the Saint-Eugène region colony is slightly lower than for the other sites.

Colonies have equivalent strength at the start. On October 25, the Montreal region colonies are significantly weaker with 5.4 bee frames, which is about one frame less than colonies in the other regions. This difference was not observed anymore on the April 7, 2006 evaluation. Colonies from the Saint-Eugène region are weaker than the others. In addition, these colonies do not develop themselves as quickly as the others because on May 15, 2006, the Montreal region colonies already were at 9 bee frames, those of the Upton regions at 8.7 frames, and those of Granby at 7.9 frames, while those of the Saint-Eugène region were weaker with 6.7 bee frames. We must highlight that the colony group from Saint-Eugène had the highest number of movements for pollination: apples, blueberries, cranberries, and vine crops.

Honey production between July and August 2005 was more important for colonies placed in the Montreal region with 18.3 kg, despite the fact that these colonies were weaker in October 2005. Colonies placed in the Upton and Saint-Eugène regions had a honey production lower than for the two other colony groups. We must highlight that colonies from these two regions are the only ones that were used for cranberry pollination.

The varroasis levels measured in September 2005, just before the fall treatment with Thymovar®, are higher in the colonies from the Montreal region. Despite a daily fall of 7.9 varroa mites at that time, these colonies reacted well to fall treatments and they came out of wintering as strong as those of the other groups, in addition to developing very well afterwards.

Acariosis levels are similar between groups. Nosema disease was not detected.

Table 4.1. Comparison of the groups having received the different summer treatments based on the protocol. The significant differences between groups are indicated by different letters (α<0.05).

Variables	Group A Montreal	Group B Saint-Eugène	Group C Upton	Group D Granby
Number of active colonies 2005-2006				
- July 26	40 (100%)	40 (100%)	40 (100%)	40 (100%)
- October 23	36 (90%)	38 (95%)	37 (93%)	39 (98%)
- April 7	32 (80%)	38 (95%)	36 (90%)	38 (95%)
Average temperature 2005 (hourly average during the month, ºC, µ±sd)				
- July	22.2 ± 1.3	22.1 ± 1.3	22.2 ± 1.3	22.2 ± 1.3
- August	23.5 ± 4.4	22.4 ± 5.0	23.8 ± 6.3	23.8 ± 4.6
- September	22.4 ± 7.5	20.5 ± 7.5	21.5 ± 6.4	21.4 ± 4.5
Pollination activities				
- Apples	**X**	**X**		**X**
- Blueberries	**X**	**X**		**X**
- Cranberries		**X**	**X**	
- Vine crops		**X**		
Hive strength (µ±sd, excl. dead bees)				
- July 26, 2005 (relative score: 1-2-3)	1.7 ± 0.1 [a]	1.9 ± 0.1 [a]	1.8 ± 0.1 [a]	1.9 ± 0.1 [a]
- October 23, 2005 (bee frames)	5.4 ± 0.2 [b]	6.4 ± 0.3 [a]	6.3 ± 0.3 [a]	6.5 ± 0.2 [a]
- April 7, 2006 (bee frames)	5.7 ± 0.2 [a]	4.6 ± 0.3 [b]	5.8 ± 0.3 [a]	5.5 ± 0.2 [a]
- April 15, 2006 (bee frames)	7.4 ± 0.3 [a]	5.2 ± 0.3 [b]	6.7 ± 0.3 [a]	5.7 ± 0.2 [b]
- May 6, 2006 (bee frames)	9.0 ± 0.2 [a]	6.7 ± 0.3 [c]	8.7 ± 0.4 [a]	7.9 ± 0.2 [b]
2005 honey production				
- Total July/September	18.3 ± 1.2 [a]	7.2 ± 0.7 [c]	6.4 ± 0.6 [c]	13.5 ± 1.0 [b]
Varroasis summer 2005 (v. mites/day)				
- September 19	7.2 ± 1.5 [a]	0.04 ± 0.02 [b]	0.03 ± 0.02 [b]	1.3 ± 0.4 [b]
Acariose 2005				
September	2.0%	1.0%	0.5%	1.5%
Nosema disease 2005				
September	Absent	Absent	Absent	Absent

Conclusions

- Climate variations between the four regions under study did not seem to influence the colonies' development and production.
- Our results seems to show an effect of pollination on the colonies' development and production: colonies that were moved for the pollination of four different crops from May to July are the weakest after the wintering period; colonies used for cranberry pollination produced less honey in August.

C. Completed Projects Part 2

5. Use of sucrose powder as summer treatment against varroasis

Introduction

A few studies are showing that the use of sucrose powder in a hive infested with varroasis is causing an intense cleaning reaction from bees. This cleaning has the effect of dislodging several varroa mites from bees and have them fall at the bottom of the hive. Sucrose powder not being a pesticide, treatment must be made on hives with an anti-varroa mite tray, thus trapping dislodged varroa mites. Given the sucrose powder's safety, its use as acaricidal treatment could be very interesting for a beekeeper with colonies infested with varroasis during the honey flow period.

Material and Method

Application of sucrose powder in the summer for controlling varroasis

The experience started at the end of July, i.e. at the end of the first honey flow. Eighteen colonies of equivalent strength were distributed in the three groups based on the Table 5.1's protocol.

Table 5.1. Research protocol and description of experimental groups for summer treatments.

Group 1	Group 2	Group 3
Control Without any treatment	Experimental 20 g sucrose powder / brood super (maximum 2 supers) 4 treatments in two weeks	Experimental 20 g sucrose powder / brood super (maximum 2 supers) 2 treatments in two weeks
N=6	N=6	N=6

All colonies were placed in the same apiary of the Centre de recherche en sciences animales de Deschambault. Sucrose powder is of food grade quality and put in the "super fine" category because of the size of the sugar particles. Our observations of particles with an optical microscope helped measuring particles that had a diameter of about 20 µm.

Sucrose powder is applied directly on bees in the brood supers (Pictures B and C). Two dosages were tested: 20 g of sucrose powder per brood super with application repeated four times between August 1 and August 15, and 20 g of sucrose powder per brood super with application repeated twice within this same period.

Pictures illustrating a colony weighing (picture A), as well as the application of the sucrose powder on bees in a brood chamber (pictures B and C).

Application of sucrose powder in the fall for controlling varroasis

The experience started in September. Fifteen colonies of equivalent strength were selected and randomly distributed in the various groups of the experimental protocol (Table 5.2). The control colony group is treated with CheckMite® based on the manufacturer's (Bayer inc.) recommendations; the other two colony groups are treated with MiteAway2® based on the manufacturer's (NOD Apiary) recommendations. In the two colony groups treated with MiteAway2®, a group is receiving a pre-wintering treatment with oxalic acid through dripping (4% in a 1:1 sucrose solution, 5 ml per frame space with bees) and the other group is

receiving an experimental treatment with sucrose powder (two 20 g applications at 7 days interval). The application of sucrose powder is done as previously described in the summer treatments.

Table 5.2: Research protocol and description of experimental groups for fall treatments.

Group 1	Group 2	Group 3
Control Coumaphos (CheckMite) September 18, 2007	Experimental MiteAway2 September 18, 2007 and Oxalic acid through dripping 35 g/L, 5 ml/frame (once)	Experimental MiteAway2 September 18, 2007 and Sucrose powder through sprinkling 20 g per brood super (2 treatments one week apart)
N=5	N=5	N=5

Dependent variables followed

- Evolution of varroasis: the level of parasitism by *Varroa destructor* is evaluated based on the natural fall method. A sticky sheet covering a third of the lower surface was placed under the frames of a colony for a minimum of five days. These data are presented in daily fall of varroa mites.
- Honey production: in kilograms.
- Evolution of the colonies' strength: the colonies' strength was evaluated from the weight (kg) or the number of frames occupied by bees in the brood chamber (1 to 10).

The statistical analyses were completed with the help of the SAS software. For variables that do not respect a normal distribution (the varroa mite fall per day variables), the initial comparison of the four groups was completed through a Kruslkall-Wallis non-parametric analysis with the NPAR1WAY procedure. If the analysis revealed a significant difference (α<0.05), a comparison of groups between them was made with a Wilcoxon test. The other variables that respected normal distribution were submitted to a variance analysis with the GLM

(generalised linear model) procedure and a LSD (least significant difference) contrast test if need be.

Results and Discussion

Application of sucrose powder in the summer for controlling varroasis

Table 5.3 is indicating results achieved during the summer tests with sucrose powder. The colonies from all three groups survived until the final evaluation at the end of August. There was no effect of the application of sucrose powder on the colonies' weight at the end of August and colonies produced similar amounts of honey in August. We observed that the application of sucrose powder at a four application dosage is causing a total varroa mite fall that is higher that for the two other groups during the treatment period (Table 5.3), but this difference is not significant ($\alpha>0.05$). In Figure 1, we can see that varroa mite fall is increasing slightly during sucrose powder application at the highest dosage (four times 15 g from August 1 to August 15). After the treatment period, daily varroa mite falls are similar between the control group and the colony group having received two sucrose applications. For the colony group having received four sucrose applications, the natural varroa mite fall is higher and even seems to increase (Figure 5.1).

Table 5.3. Effect of the application of sucrose powder in August after the first honey flow. The different lowercase letters for a variable's data (lines) indicate a significant difference ($p<0.5$).

Variables	Group 1: Control Without treatment	Group 2: Experimental 4 applications of powdered sugar	Group 3: Experimental 2 applications of powdered sugar
Number of active colonies - July 31, 2005 - August 28, 2005	 6 6	 6 6	 6 6
Hive strength - July 29, 2005 (weight in kg µ±sd) - August 27, 2005 (weight in kg µ±sd)	 71.7 ± 3.3 [a] 84.3 ± 5.8 [a]	 72.1 ± 3.9 [a] 85.1 ± 4.4 [a]	 77.4 ± 5.5 [a] 88.1 ± 9.5 [a]
Honey production - b/w July 29 and August 27, 2005	 12.2 ± 3.6 [a]	 12.9 ± 1.7 [a]	 10.4 ± 4.8 [a]
Varroasis Natural varroa mite fall (total number of varroa mites on a sticky sheet) during the treatment period (b/w August 2-20, 2005)	 18.0 ± 2.8 [a]	 39.0 ± 21.6 [a]	 8.0 ± 2.3 [a]

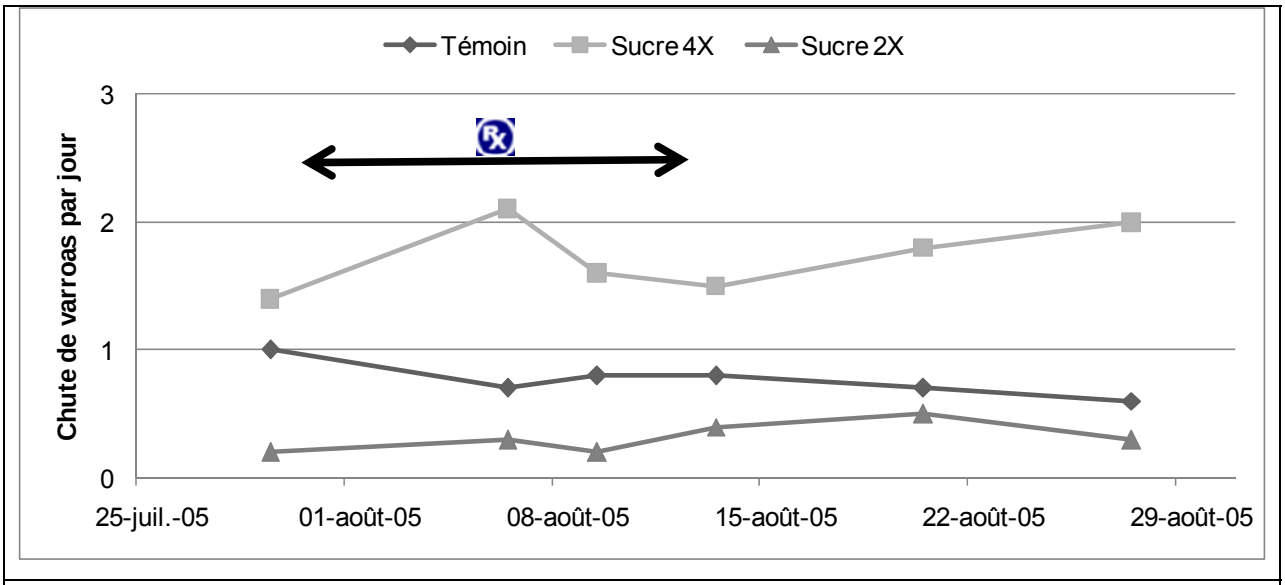


Figure 5.1. Evolution of the natural varroa mite fall between July 29, 2005 and August 27, 2005. The application of sucrose powder began on August 2. The control group did not receive any treatment against varroasis, while the two other groups received either four applications or two applications of sucrose powder until August 16, 2005.

Application of sucrose powder in the fall for controlling varroasis

Table 5.4 is indicating results achieved during the fall tests with sucrose powder application. All colonies from the three groups survived following wintering. The first mortalities were observed after colonies were taken out of the repository. The colonies' strength is similar between groups in the October and November evaluations. In the spring, colonies treated with CheckMite® had a brood number that was significantly inferior ($\alpha<0.05$) than the other two groups, despite a similar bee frame evaluation. The varroa mite fall during treatments with oxalic acid is significantly superior than the one measured during the sucrose powder treatment. The following spring, a global evaluation of the effectiveness of all three tested treatments indicated that the treatment with MiteAway2® combined with a sucrose powder application at the end of fall resulted in an effectiveness that is significantly inferior to the treatment with MiteAway2® and oxalic acid, as well as the control treatment with CheckMite®.

Table 5.4. Comparison between the application of sucrose powder and oxalic acid at the end of fall just before the wintering period in a repository. The different lowercase letters for a variable's data (lines) indicate a significant difference ($p<0.5$).

Variables	Group 1: Control Coumaphos (CheckMite)	Group 2: Experimental MiteAway2 + Oxalic acid	Group 3: Experimental MiteAway2 + Powdered sugar
Number of active colonies			
- August 31, 2007	8	6	6
- November 14, 2007 (entered repository)	8	6	6
- April 21, 2008 (out of repository)	8	6	6
- May 13, 2008 (end of test)	6	5	5
Hive strength (excluding dead bees)			
- Oct 31, 2007 (bee frames µ±sd)	6.3 ± 0.4 [a]	7.0 ± 0.3 [a]	7.0 ± 0.2 [a]
- November 12, 2007 (weight in kg µ±sd)	40.3 ± 1.4 [a]	37.8 ± 0.6 [a]	37.3 ± 1.3 [a]
- April 21, 2008 (weight in kg µ±sd)	27.7 ± 1.6 [a]	27.4 ± 1.2 a	27.5 ± 0.8 [a]
- May 1, 2008			
Frames/bees µ±sd	6.2 ± 1.0 [a]	6.7 ± 0.9 [a]	6.0 ± 0.5 [a]
Cells/brood µ±sd	5925 ± 1080 [a]	14540 ± 1746 [b]	13883 ± 2157 [b]
Varroasis			
Total natural varroa mite fall during treatment with oxalic acid and sucrose powder (from November 1 to November 14, 2007)		493.2 ± 105.1[a]	84.7 ± 25.1[b]
Total effectiveness (reduction % between August 31, 2007 and May 1, 2008)	90.6 ± 4.6[b]	87.0 ± 7.7[b]	73.3 ± 7.7[a]

Conclusions

- Sucrose powder application in the summer or fall does not allow to significantly reduce the infestation levels of honeybee colonies with *V. destructor*.
- Sucrose powder application seems to cause the fall of a certain number of varroa mites; however, the effect on varroasis evolution is negligible.

6. Spring treatments against varroasis and acariosis

Abstract

The goal of this research was to measure the effectiveness of organic acids against varroasis in the spring. Forty-eight hives infested with varroasis (natural fall < 1 varroa mite a day in early May) were randomly distributed into eight experimental groups submitted to the following treatments: two dosages of oxalic acid through dripping (oxalic acid 40 g/L of 1:1 sugar syrup, two 5 ml applications per frame space at seven days interval and a 10 ml application per frame space), three formic acid application methods (Mitewipe, ad hoc application -'Flash' and MiteAway2®) and a control group without treatment.

Results are showing that the growth of the varroa mite population in colonies of each group is similar (α>0.05) following treatments. This indicates that an anti-varroa mite treatment is not useful in the spring when infestation levels are below 1 varroa mite/day (natural fall). However, results are showing that acid treatments had an impact on colonies. The number of bees that died during treatments is higher in the groups treated with formic acid. During the evaluation after treatments (June), the control group had more bees and brood than the group treated with MiteAway2® (α<0.05). At the end of the season (September), the brood number is lower in the group treated with MiteAway2® and higher in the group treated with a dose of 10 ml of oxalic acid (α<0.05). Honey harvest is lower in the group treated with MiteAway2® and higher in the control group and the group treated with oxalic acid (two 5 ml dosages at seven days interval). Analyses made on honey harvested in mid-season in each of the experimental groups show that the concentrations of both acid types in honey are similar to those measured in the control group.

Material and Method

Preparation of hives and experimental protocol

The bee colonies used belong to the Centre de recherche en science animale de Deschambault (CRSAD). All 48 hives spent the winter in a repository. They received a formic acid treatment on September 25, 2004, followed with a second treatment, towards mid-November, with oxalic acid through dripping (40 g/L in a 1:1 sugar solution, 5 ml per frame space). After being taken out of the repository in the spring of 2005, hives were weighed and their bee population was evaluated. Forty-eight hives of equivalent strength and varroa mite infestation level were randomly distributed in the protocol's six experimental groups (Table 6.1).

Table 6.1. Research protocol and description of the different treatments completed in the spring of 2005.

Group 1 Control	**Group 2 Experimental**	**Group 3 Experimental**	**Group 4 Experimental**	**Group 5 Experimental**	**Group 6 Experimental**
Without treatment	Oxalic acid Dripping 40g acid /L sugar syrup (1:1) 10 ml/bee frame 1 treatment	Oxalic acid Dripping 40g acid /L sugar syrup (1:1) 5 ml/bee frame 2 treatments at 7 days interval	65% formic acid 1 Mitewipe 35 ml per super 2 applications at 7 days interval	65% formic acid Ad hoc 2 ml per bee frame 2 applications at 7 days interval	65% formic acid MiteAway2® Application per label
N=8	N=8	N=8	N=8	N=8	N=8

Treatment sequence

Treatments started on May 10 with the application of all treatments based on the protocol. After seven days, experimental groups 3, 4, and 5 received a second application as planned. During this period, all hives were at one super. From May 31, an additional super was added to strong hives and, afterwards, colonies were integrated in the normal management of the CRSAD's bee herd until the end of the summer.

Starting September 27, all colonies were submitted to a CheckMite® (coumaphos) control treatment.

Application of oxalic acid (OA)

The dripping method was selected for the experimentation. For this application, we are using a solution consisting of 40 g of dihydrated oxalic acid diluted in 1 litre of sugar syrup (1:1). This solution is poured between frames and directly on bees with a Brinkmann Dispensette pump (Figure 6.1).

A

B

Figures 6.1 A and B. Oxalic acid application through dripping (40 g/L solution, in a 1:1 sugar solution)

Application of formic acid (FA)

- MiteAway2® (Figure 6.2A): Formic acid is first incorporated in a 10 x 10 x 0.7 cm pad. This commercial product is sold in an airtight bag containing a pad soaked with 250 ml of formic acid covered with plastic with a perforated side. The MiteAway2® pad must be placed on top of frames at the back of the hive with perforations towards the bottom. The hive cover must be raised by installing a 2-cm separator. The spring

treatment's recommended duration is 21 days for a hive with a single super.

A

B

C

D

Figure 6.2. A) MiteAway II® application, B) Mitewipe swaps; and C) Ad hoc ('Flash') method. D) Insertion of a Gastec® tube to measure the formic acid concentration in the colony.

- Mitewipe (Figure 6.2B): Formic acid is soaked in an absorbent paper put in a plastic film with a perforated side (Mitewipe pads). Each pad is soaked with 35 ml of formic acid. Mitewipe application is done by putting the perforated side on the top of the brood super's frames.
- Flash (figure 6.2C): Formic acid is put directly on the hive's anti-varroa mite tray. Acid dripping is avoided with towelling paper.
- The evolution of the formic acid concentration was measured in two colonies taken at random for each acid application methods. Concentrations (ppm) in these colonies were determined through the use of Gastec® tubes for organic acid (Figure 6.2D).

Dependent variables measured

- Varroa mite fall: Each hive contains an anti-varroa mite tray in which a sticky sheet can be put (full length of the base) to collect varroa mites falling from bees. The varroa mite fall on sticky sheets was measured at the following dates:
    - Pretreatment: April 19-26, 2005;
    - During treatment: May 10 to June 7: sheets changed on the 7th day;
    - Post-treatment: June 7-14, June 14-21, July 5-12, August 8-15, September 6-13;
    - Fall control with MiteAway2®: September 27-October 11;
- Evolution of brood and bee populations: The bee population of each hive was assessed by measuring the area occupied by the hives' brood and by comparing the area occupied by bees with similar pictures with an already-measured bee number. This evaluation was carried out before and after treatments.
- Bee mortality during treatment: An estimate of bee mortality was completed during treatment by counting bees collected on a 1 meter square piece of geotextile placed at the hive's entrance. This estimate was made every day from May 10-30.
- Hive's honey gain: Hives were weighed as soon as they were taken out of the repository, before and after treatments, as well as during every addition and withdrawal of honey supers. This way, honey stocked by bees was counted during the entire season.
- Oxalic and formic acids' residues in harvested honey: At the end of June, the hives' honey was grouped together per experimental group. We took two 50 g honey samples from each honey batch from the various groups. These samples were kept in airtight containers in a freezer (-20°C) until it was time to analyze them. Formic acid and oxalic acid were dosed after an extraction with 0.2 N sulphuric acid. These organic acids were separated and quantified with a Waters (Waters corporation, Massachusetts, USA) high-performance liquid chromatography (HPLC)

system, equipped with an AMINEX HPX-87H (7.8 mm DI X 30 cm) (Bio-Rad laboratories California USA) separation column. The mobile phase consisted of 0.0025 N sulphuric acid with a 0.4 ml/min flow and a temperature of 40°C. Organic acid detection was made with a refractometer (Waters 410). These analyses were made based on the MAPAQ's procedure[1].

Statistical analyses

Data was analyzed through the use of the GLM (general linear model) procedure of the SAS (statistical analysis system) software. Contrasts between the different experimental groups were obtained through the LSD (least significant difference) method.

[1] MINISTERE DE L'AGRICULTURE, DES PÊCHERIES ET DE L'ALIMENTATION. Détermination du taux d'acide oxalique et d'acide formique dans le miel et la cire d'abeille, MA. AcOx 1.0, Ministère de l'agriculture, des pêcheries et de l'alimentation, 2003.

Results

Varroa mite fall

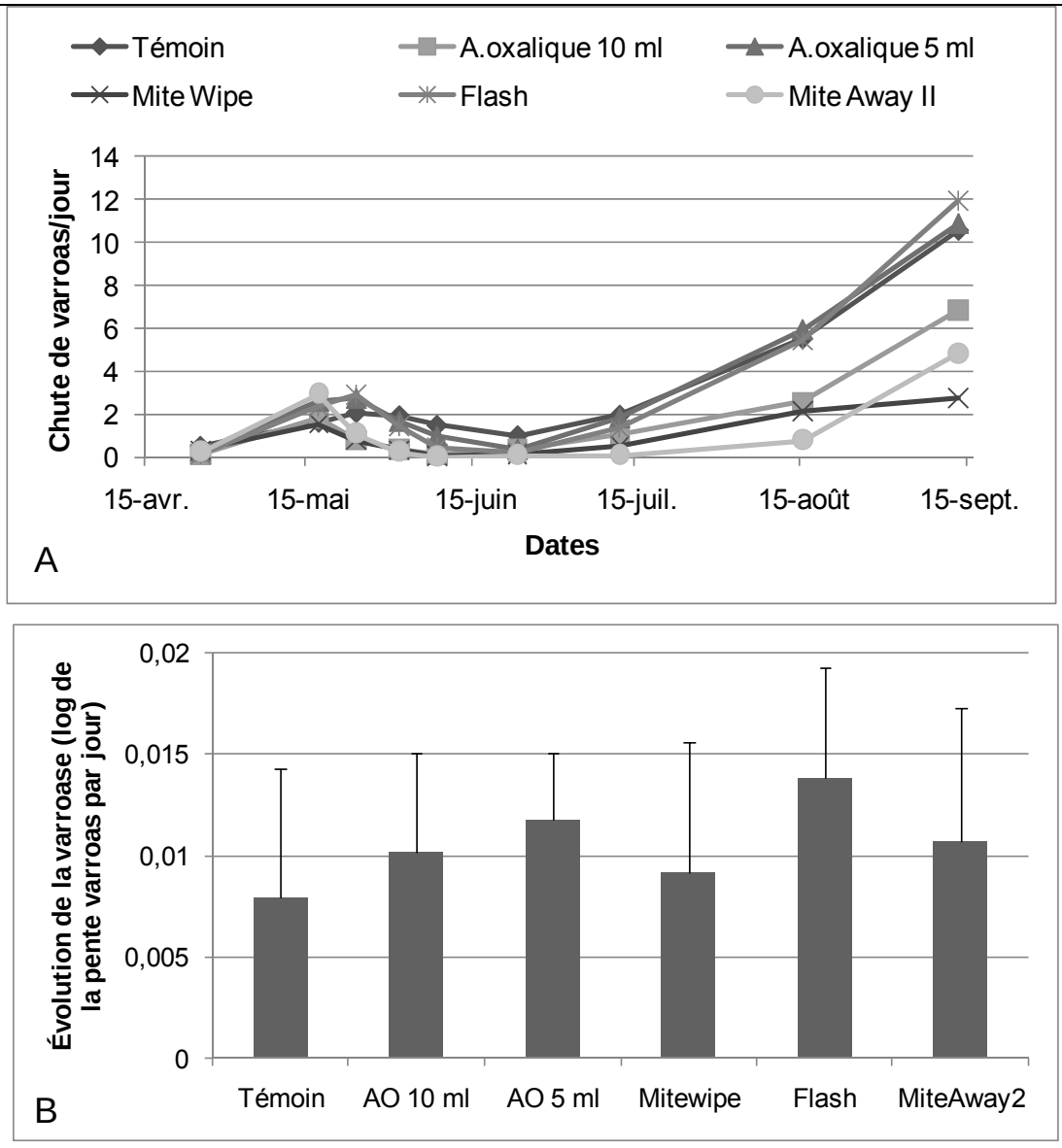


Figure 6.3. A) Evolution on varroa mite fall during the 2005 season. Spring treatments started on May 17 and ended on June 8. The CheckMite™ treatment started on September 15. B) The growth levels expressed in log of the daily varroa mite fall per colony during the post-treatment period are not significantly different between experimental groups (α=0.46 respectively, ANOVA, LSD Test).

The natural varroa mite fall average for colonies in the various experimental groups was lower than one varroa mite a day before treatments began. Treatments caused a slight increase of the natural fall (May 15 to June 8, Figure 6.3A) during the first two weeks in all groups. This fall is slightly superior in the three groups treated with formic acid. Afterwards, the natural varroa mite fall growth is similar (comparison of the varroa mites/days ln slope, $\alpha>0.05$) in all experimental groups and the control group that did not receive any treatment (Figure 6.3B).

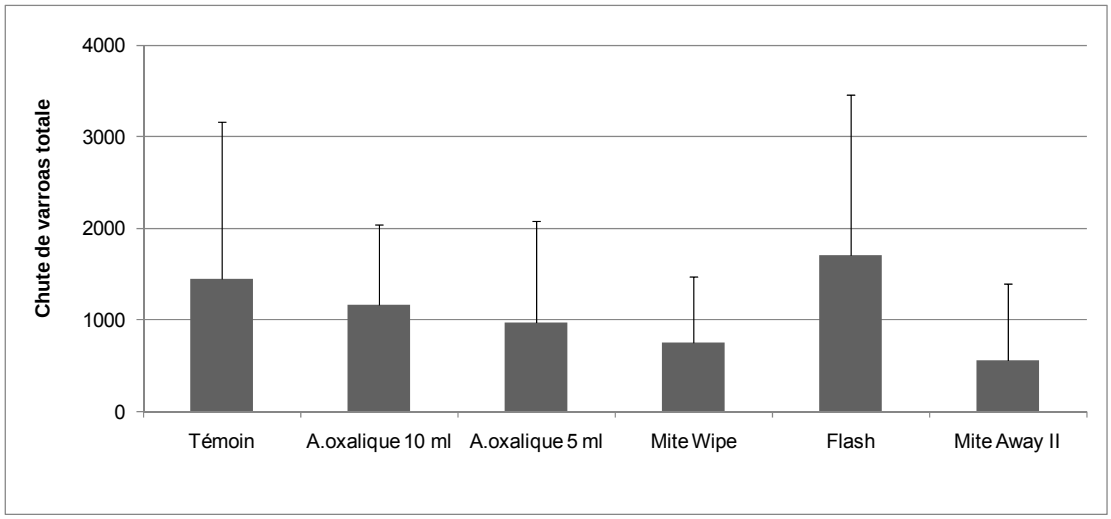


Figure 6.4. Total number of varroa mites counted on sticky sheets during the CheckMite™ control treatment from September 27 to October 11, 2005. There are no significant differences ($\alpha>0.05$) between groups when isolating the apiary variable (variance analysis by adding the apiary variable in the model).

During the control treatment in the fall (Figure 6.4), the total number of varroa mites in colonies of the different groups varies between 600 and 1800 without there being significant differences. Varroa mite populations in the different colonies of each experimental group are very variable.

Colony and queen survival

There were queens dead only during the experimental treatments with formic acid. Two queens died with MiteAway2® and Flash and one queen died in the Mitewipe group. All other colonies kept an active queen until the CheckMite® control treatment in September.

Evolution of the brood and bee populations

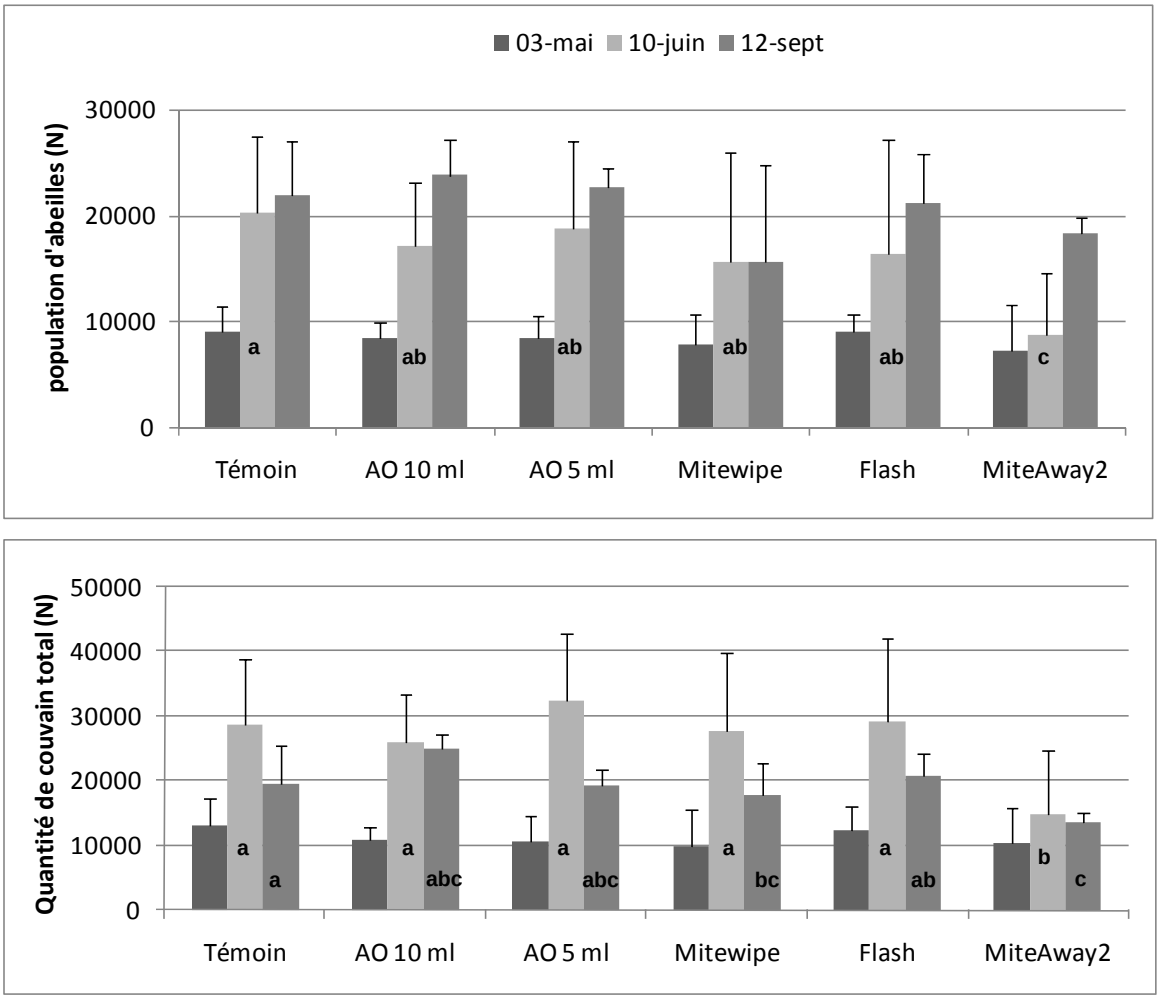


Figure 6.5. Comparison of the bee (A) et brood (B) population in colonies before (May 3) the spring treatments and after (June 10). Values represented by columns of the same colour with different letters indicate a significant difference ($\alpha < 0.05$ ANOVA, LSD Test).

Figures 6.5A and B are showing the evolution of the bee and brood population in colonies of the different experimental groups and in the control group. After treatments (June 10), the number of bees was higher in the control group and lower in the MiteAway2® group ($\alpha<0.05$). The other groups' averages are mid-way between these two values. During the September evaluation, the average bee number per colony was similar in all groups.

After treatments (10 June), the number of brood per colony is lower in the MiteAway2® group as opposed to all other groups ($\alpha<0.05$). During the September evaluation, the MiteAway2® group was still the group with the lowest average brood number ($\alpha<0.05$), while all other groups had similar values.

Bee mortality during treatment

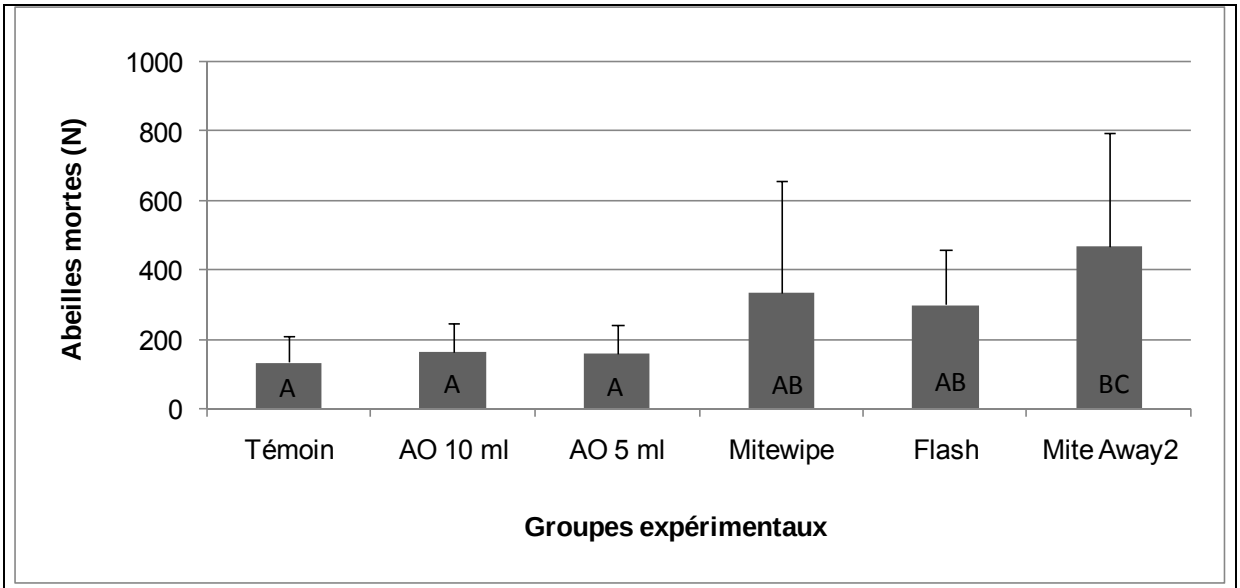


Figure 6.6. Bee mortality (daily mean ± standard deviation) during treatments (from May 17 to May 30, 2005). Columns with different letters indicate a significant difference (< 0.05 ANOVA, LSD Test).

The average daily mortality per colony (Figure 6.6) is higher in the three groups that received formic acid. The MiteAway2® group had superior mortality that significantly distinguished itself from the control group colonies and colonies

treated with oxalic acid through dripping (α< 0.05). The mortality of the two colony groups that received oxalic acid treatments was equivalent to that of the control group.

Hives' honey gain

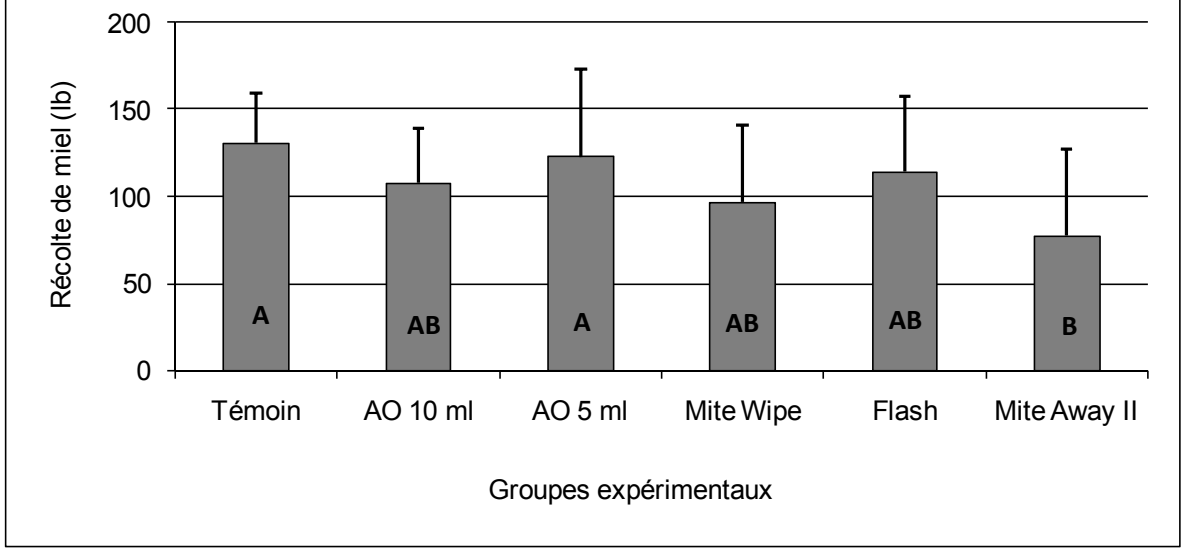


Figure 6.7. Honey harvest (mean ± standard deviation) in the different experimental groups during the 2005 season. Columns with different letters indicate a significant difference (< 0.05 ANOVA, LSD Test).

Hives in the control group and hives treated with 5 ml of OA had the best honey gains during the May to September harvest season (Figure 6.7). The MiteAway2® group colonies collected the least amount of honey with an average of 77 kg during the season and this value is significantly lower than the control group and 5 ml of OA (α< 0.05).

Oxalic and formic acids' residues in harvested honey

Table 6.2 is showing the concentrations (ppm) of oxalic and formic acids in honey samples collected during the end-of-June extraction. Results indicate that

there is no difference between honey from colonies treated in the spring and the control group colonies.

Table 6.2**.** Concentrations of oxalic acid and formic acid in harvested honey. Honey was extracted from all colonies and grouped together per experimental group. Two 50 ml honey samples were analyzed for each experimental group.

Experimental group	Oxalic acid (ppm)		Formic acid (ppm)	
	sample1	sample2	sample1	sample2
GROUP 1: Control w/o treatment	22	22	0	0
GROUP 2: Oxalic acid, 40 g/l sugar syrup (1:1) 10 ml/frame; 1 application	21	23	0	0
GROUP 3: Oxalic acid, 40 g/l sugar syrup (1:1) 5 ml/frame; 2 applications (7 days)	22	23	0	0
GROUP 4: 65% formic acid 1 Mitewipe 35 ml; 2 applications (7 days)	20	22	0	0
GROUP 5: 65% formic acid Ad hoc 2 ml/bee frame (Flash) 2 applications	22	23	0	0
GROUP 6: 65% formic acid MiteAway II®	22	23	0	0

Discussion

Effectiveness of experimental treatments

The different spring treatments tested did not demonstrate significant differences in their capacity of reducing varroa mites in colonies. All groups had a similar growth level for daily natural varroa mite fall in the summer (June to September) and a similar number of varroa mites in September (CheckMite® control treatment). In addition, these values are not significantly different that those measured for the control group. We come to the conclusion that in a situation of low infestation level in the spring (natural fall $<$ 1 varroa mite a day), the suggested treatments are not necessary. However, results of our work enable us to measure the effect of these spring treatments on productivity and colony growth during the beekeeping season.

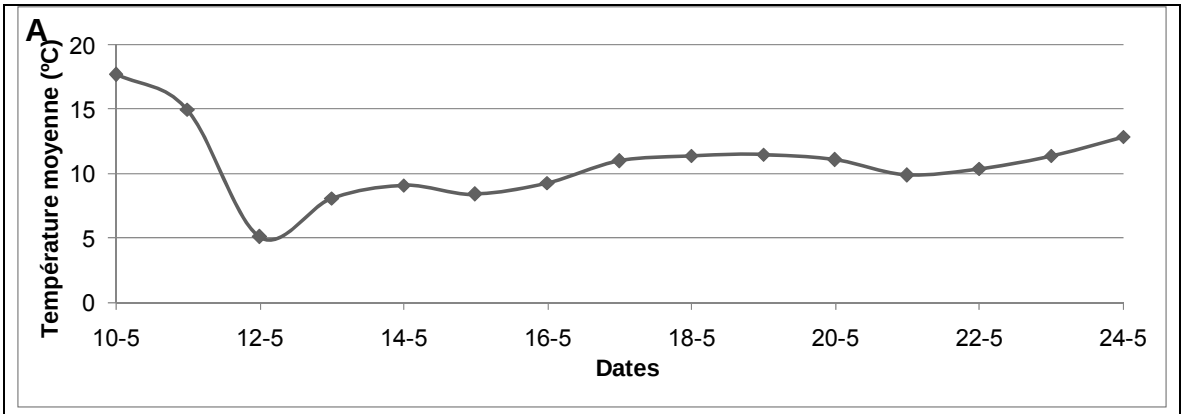
Effect of experimental treatments on hives

The toxicity of formic acid applied in the spring for bees is demonstrated through our results. Thus, the colonies of the three groups that received formic acid treatments had a higher number of dead bees than colonies of the control group and groups treated with oxalic acid through dripping. The toxic effect of formic acid on brood is more pronounced in the colony group treated with MiteAway2®. The impact of this type of formic acid application in the spring is having repercussions all along the season. A beekeeper working with this product in the spring must therefore make sure that their colonies are strong enough to support such a treatment. It may even be better to wait a few weeks to let colonies reach a certain development level before using MiteAway2®.

The use of oxalic acid had no harmful effects on bees and their brood. Despite the fact that this product has no effect on varroa mites in the brood, we believe that repeating its application on a seven-day period could be an interesting alternative in the spring treatment, given its low toxicity with bees.

All tested treatments in this protocol did not cause the accumulation of residues in honey. We can thus come to the conclusion that these spring treatments do not influence honey's safety.

Formic acid concentration in hives during treatments



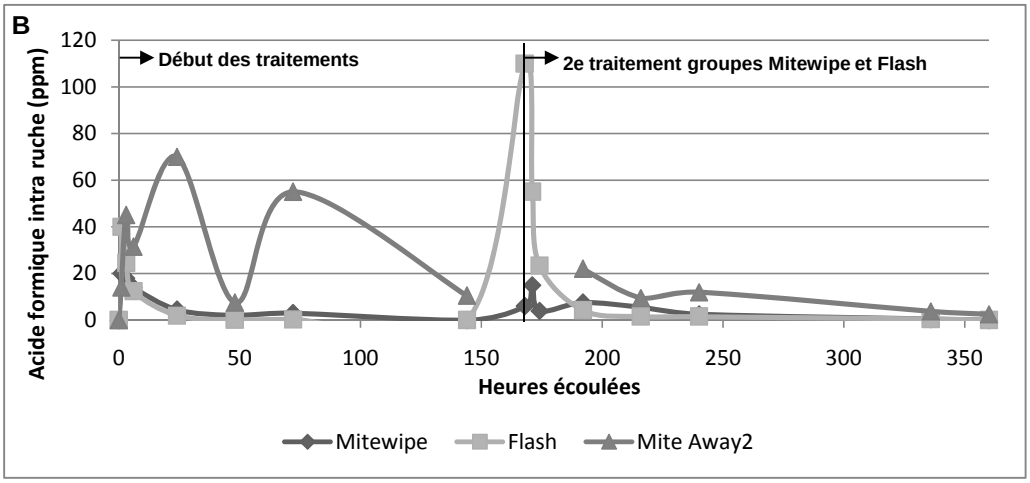


Figure 6.8. A) Evolution of daily temperature (average of 24 hourly measurements per day) measured on the experimental hive's site from the beginning of treatment application until the fourteenth day. B) Evolution of formic acid concentration (average of two hives per treatment, ppm) in colonies of experimental groups 4, 5, and 6 during the first fourteen days of the protocol. Measurements were taken after acid application (1 hour, 3 hours, and 6 hours) and afterwards at one day or several day periods.

The three formic acid application methods are showing different concentration evolution profiles (Figure 6.8). MiteAway2® is spreading the highest concentrations during the first application days, which then gradually decrease. In addition, our measurements indicate that the spreading of formic acid with

MiteAway2® depends on the outside temperature: a drop in outside temperature measured two days after application had measurable repercussions on the formic acid concentration in colonies. For the other two application methods, acid evaporation is much quicker and completed after 48 hours.

Conclusions

- It is not worthwhile to proceed with a spring treatment when varroa mite falls are inferior to one a day.
- Formic acid treatments or a 10 ml dose of oxalic acid per frame space (dripping) are disrupting spring colonies enough to have significant repercussions on the hive's population and on honey production during the summer.
- The beekeeper must aim at a successful fall treatment. He will thus avoid the spring treatment on growing colonies and colonies sensitive to acaricide treatments using oxalic and formic acids.

7. Evaluation of two oxalic acid application methods before hive wintering

Goal

- To measure the effectiveness of different oxalic acid application methods against varroasis before wintering and to compare the effects on the development of the bee colonies the following spring.

Material and Methods

The research work began at the end of August. Twenty honeybee colonies of similar strength and with equivalent natural varroa mite fall levels were randomly distributed in the two groups of the experimental protocol illustrated in Table 7.1. In September, all colonies were treated with MiteAway2® based on the manufacturer's (NOD Apiaries inc.) recommendations. Afterwards, both groups received two types of oxalic acid applications: the group 1 colonies received oxalic acid through dripping (4% in a 1:1 sucrose solution, 5 ml per frame space with bees), while the group 2 colonies received oxalic acid through thermal sublimation with the Varrox® (Andermatt Biovet AG) unit at a dosage of 2 g of acid per colony full of bees.

The dependent variables that are followed are:

- Evolution of varroasis: the level of parasitism by *Varroa destructor* is evaluated based on the natural fall method. A sticky sheet covering a third of the lower surface was placed under the frames of a colony for a minimum of five days. These data are presented in daily varroa mite fall and in daily fall reduction percentage.
- Evolution of the colonies' strength: the colonies' strength was evaluated from the weight (kg) or the number of frames occupied by bees in the brood chamber (1 to 10).

Table 7.1. Research protocol and description of the different fall treatments.

Group 1	Group 2
Experimental MiteAway2 in September Oxalic acid through dripping 1:1 Sugar syrup with 40 g/L of oxalic acid 5 ml/between bee frame	Experimental MiteAway2 in September Oxalic acid through thermal sublimation Varrox unit 2 g of oxalic acid per hive with one super
N=10	N=10

The statistical analyses were completed with the help of the SAS software. For variables that do not respect a normal distribution (the varroa mite fall per day variables), the initial comparison of the four groups was completed through a Wilcoxon non-parametric analysis with the NPAR1WAY procedure. The other variables that respected normal distribution were submitted to a variance analysis with the GLM (generalised linear model) procedure.

Results and Discussion

Table 7.2 is indicating results achieved. No colony died during this protocol (October to May). Results are showing that colonies that received the oxalic acid treatment through dripping are stronger the following spring: these colonies had, in average, 6.6 bee frames, i.e. 1.1 frames more that colonies treated with acid through thermal sublimation. This result has an impact on the colonies' spring resumption because during the brood's detailed evaluation competed two weeks later (May 5), the stronger colonies in group 1 had an average of 17,350 brood cells (eggs and larvae), while those in group 2 had 14,904 brood cells ($\alpha<0.05$).

Table 7.2. Comparisons of the two oxalic acid application methods before wintering in a repository. The different lowercase letters for a variable's data (lines) indicate a significant difference (α<0.5).

Variables	Oxalic acid through dripping (For the treatments' description, see Table 1)	Oxalic acid through sublimation (For the treatments' description, see Table 1)
Number of active colonies		
- October 11, 2005	23 (100%)	23 (100%)
- November 18, 2005 (in the repository)	23 (100%)	23 (100%)
- April 19, 2006 (out of the repository)	23 (100%)	23 (100%)
- May 1, 2006 (end of test)	23 (100%)	23 (100%)
Hive strength (excluding dead bees)		
- Nov. 18, 2005 (weight in kg µ±sd)	38.4 ± 0.4 [a]	38.5 ± 0.8 [a]
- April 19, 2006		
Weight in kg µ±sd	26.8 ± 0.7 [a]	27.6 ± 0.6 [a]
frames/bees µ±sd	6.6 ± 0.3 [a]	5.5 ± 0.4 [b]
- May 5, 2006 (cells/brood µ±sd)	17350 ± 1046 [a]	14904 ± 996 [b]
Varroasis		
Treatment effectiveness (Reduction % between Oct. 11 and Dec. 21)	96.3 ± 2.1 [a]	99.6 ± 0.3 [a]
Natural varroa mite fall/day May 1, 2006	1.2 ± 0.5 [a]	0.4 ± 0.2 [a]

The evolution of varroa mite fall (Figure 7.1) indicates that the application of oxalic acid through dripping or thermal sublimation is causing a varroa mite fall. After treatments, varroa mite falls are low and even inferior to those measured before the acid application. A measurement of the natural fall reduction level (Table 7.2) between October 11 and December 21 shows that the two application methods had similar effectiveness. However, the natural fall measured the following spring (May 1) is significantly higher in colonies treated through sublimation.

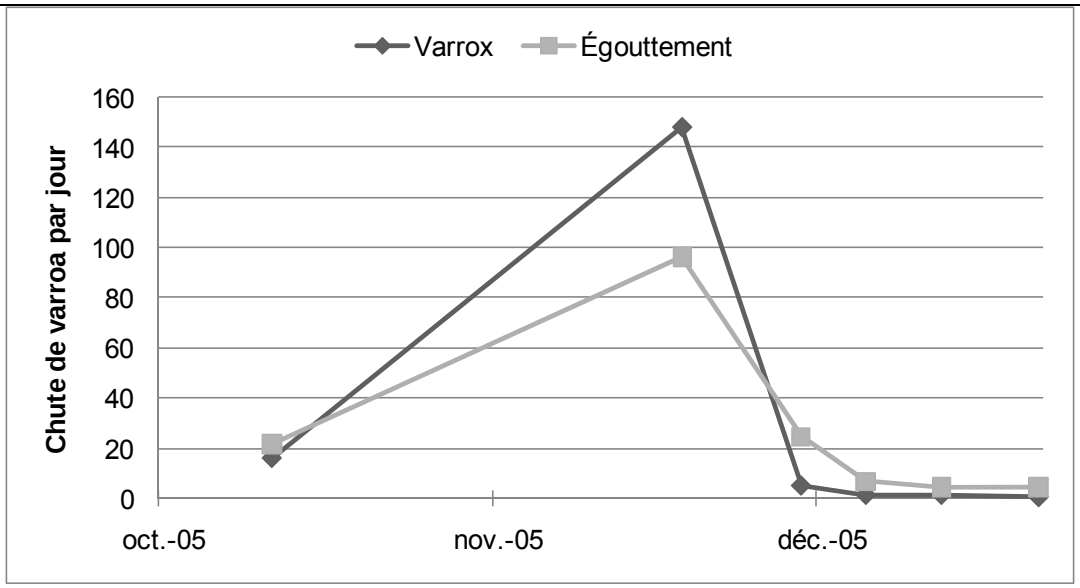


Figure 7.1. Daily varroa mite fall (mean standard error) calculated from 7 days of sampling in the fall and spring.

Conclusions

- End-of-fall treatments with oxalic acid through dripping or thermal sublimation cause a considerable varroa mite fall. They are thus effective control methods against varroasis when application is made just before wintering.
- Within our work, the application of oxalic acid through sublimation had a significant negative impact on the colonies' strength the following spring as opposed to colonies treated with oxalic acid through dripping.

8. Evaluation of an integrated pest management strategy in association with intervention thresholds

Abstract[1]

Our research goal was to test two different integrated pest management strategies that combine the use of organic pesticides (formic acid, oxalic acid and thymol) and mite economic level thresholds. In spring 2006, forty eight colonies of equivalent strength were selected. These hives survived wintering without receiving a fall varroa treatment and therefore varroa infestation rates were high and variable (7 ± 11 mite fall/day). The genetic bee stock was a mixture of Primorsky and local Italian *Apis melifera*. Hives were randomly distributed in experimental groups (Table). The oxalic acid solution (40g/litre in a 1:1 sugar solution) was dripped between each frame of bees (5ml/frame). Each Mitewipe contained 35 ml of 65% formic acid and two pads were placed on top of frames of the second brood chamber. The MiteAway® and the Apigard® treatments were applied by the label recommendations. Hives were followed for a year. Statistical data analysis was done using the ANOVA procedure with the SAS software.

Table. Integrated pest management strategies tested.

	Threshold	IPM1	IPM2
Spring	1	MiteAwayII®	Oxalic acid (2x/7days interval)
Summer	11	Mitewipe (3x/3days interval)	Oxalic acid (2x/7days interval)
Autumn 1	11	MiteAwayII®	Apigard®
Autumn 2	1	Oxalic acid (1x)	Oxalic acid (1x)

[1] Giovenazzo, P.and P. Dubreuil. INTEGRATED PEST MANAGEMENT AGAINST *VARROA DESTRUCTOR* : TREATMENT THRESHOLDS AND EFFICACY OF THE COMBINED USE OF FORMIC ACID, OXALIC ACID AND THYMOL. Proceedings of the American Bee research Conference. American Bee Journal. June 2008.

Spring treatments in both IPM strategies are inefficient. Daily mite fall increased from mid May to end of July (8.1 ± 3.0 (mean ± standard error) to 17.8 ± 3.0 in IPM1 and from 5.7 ± 1.4 to 21.3 ± 6.6 in IPM2). Mid summer treatments with Mitewipes (2 pads/3x/3days interval) reduced mite fall from 17.4 ± 3,0 mites/day to 12.0 ± 1.8 mites/day. Oxalic acid was inefficient during the same time period: varroa mite fall went from 21.3 ± 6.6 mites/day to 25.6 ± 6.8 mites/day.

Autumn treatments are the most efficient in reducing mite populations and there was no significant difference between strategies from September to November. Analysis of honey (2 samples from total honey harvested in each experimental group) from hives IPM2 showed the presence of formic acid residues (July honey=330 ppm and 317 ppm; September honey=127 ppm and 113 ppm). From May to September the bee and brood numbers and the honey yield were similar between groups. In November 2006 hives were significantly stronger in the IPM1 group (frames of bees: IPM1=8.2 ± 0.2 > IPM2=5.0 ± 0.6; $\alpha<0.01$), a similar result was also measured in May 2007 (frames of bees: IPM1=7.1 ± 0.4 > IPM2=3.4 ± 0.5; $\alpha<0.01$). We suggest that the high varroa levels measured in the IPM2 hives at the beginning of September (25.6 ± 27.3 mites/day) were mainly responsible for the low colony strenghts measured before and after wintering. The Apigard treatment reduced mite populations with good efficacy (92%) but the physiology of the wintering bees had already been affected by the high September mite load. As conclusion, we suggest that a good varroa control strategy must aim an optimal fall treatment in order to avoid a spring treatment. A mid summer treatment with Mitewipes (2 pads/3x/3 days interval) will reduce mite populations but care must be taken to avoid formic acid residues in honey.

Introduction

The goal of this protocol was to test two integrated pest management strategies against varroasis based on intervention thresholds (natural fall of varroa mites in the spring, summer and fall) determined by the work done by Chapleau (2005), Dubreuil, and Giovenazzo (2005). Pesticides used are organic acaricides (formic

acid, oxalic acid, and thymol) that gave the best effectiveness results during the different treatment and application tests in the spring, summer and fall since 2002 (Dubreuil and Giovenazzo, unpublished preliminary tests).

Material and Method

Colonies and protocol

In the spring of 2006, 48 CRSAD hives infested with varroasis were selected for this protocol. These colonies came from a genetic mix of Primorsky lines and *Apis mellifera lingustica* local Italian bee lines. After being taken out of wintering, the bee and brood population, as well as the parasitism level were evaluated. The selected hives had equivalent strength. These colonies did not receive any anti-varroa mite treatment the previous fall, so varroasis levels were high and variable between colonies (natural fall of 7 ± 11 varroa mites a day). Colonies were randomly distributed in the protocol's experimental groups (Table 8.1). Each experimental group is corresponding to a complete integrated pest management strategy (IPM), i.e. with intervention thresholds and different treatments in the spring, summer, and fall. Selected colonies were placed in equal numbers in two experimental hives of the Quebec region (Neuville and Saint-Augustin).

Treatment application

The oxalic acid solution (40 g/litre in a 1:1 saccharose solution) is poured freely between each frame with bees (5 ml/frame). Each Mitewipe pad was soaked with 35 ml of formic acid (65%) and two pads were placed on the frames of the second brood chamber (superior). MiteAway® and Apiguard® treatments were applied based on manufacturers' recommendations (NOD Apiary Products Ltd. and Vita Europe Ltd.). The natural varroa mite fall is evaluated in all hives on a seven day period during the four periods described in Table 1. When the natural varroa mite fall of a hive is reaching a threshold, it is treated with the acaricide corresponding to the period and strategy.

Table 8.1. Experimental protocol and description of two tested integrated pest management strategies (IPM), as well as intervention thresholds that were followed.

Periods	Intervention thresholds	Strategy 1 (IPM1)	Strategy 2 (IPM2)
Spring (May-June)	1 varroa mite/day	**Formic acid Mite Away 2** 21 days	**Oxalic acid Dripping** 40 g/L, 5 ml/frame 2 treatments at 7 days interval
Summer (July)	11 varroa mites a day	**Formic acid Mitewipe** 65%, two 35 ml pads 3 treatments at 3 days interval	**Oxalic acid Dripping** 40 g/L, 5 ml/frame 2 treatments at 7 days interval
Fall 1 (September)	11 varroa mites a day	**Formic acid Mite Away 2** 42 days	**Thymol Apiguard**
Fall 2 (November)	1 varroa mite a day	**Oxalic acid Dripping** 40 g/L, 5 ml/frame	**Oxalic acid Dripping** 40 g/L, 5 ml/frame

Oxalic and formic acids' residues in harvested honey

At the end of July and start of September, honey harvest from hives was grouped together per experimental group. We took two 50 g honey samples from each honey batch from the various experimental groups. These samples were kept in airtight containers in a freezer (-20°C) until it was time to analyze them (maximum six months). Formic acid and oxalic acid were dosed after an extraction with 0.2 N sulphuric acid. These organic acids were separated and quantified with a Waters (Waters corporation, Massachusetts, USA) high-performance liquid chromatography (HPLC) system, equipped with an AMINEX HPX-87H (7.8 mm DI X 30 cm) (Bio-Rad laboratories California USA) separation column. The mobile phase consisted of 0.0025 N sulphuric acid with a 0.4 ml/min flow and a temperature of 40°C. Organic acid detection was made with a

refractometer (Waters 410). These analyses were made based on the MAPAQ's procedure[2].

Nosema disease and acariosis

Bees were collected in each of the experimental groups and in each of the hives to evaluate the presence of the *Nosema sp.* and *Acarapis woodi* pathogens. Each sample consisted of a bee mix (20 bees/colonies taken in all colonies) of each experimental group of an apiary in a jar containing 500 ml of 70% ethanol. In a lab, the abdomen and thorax of 60 bees of each sample were analyzed for the presence of these pathogens[3]. Results are presented in % of bees parasitized by *A.woodi* in collected samples. This sampling was made in September 2007 (6 samples: one per experimental group in each apiary) and in April 2008 (6 samples: one per experimental group in each apiary).

List of dependent variables

- Infestation level by *Varroa destructor* during the four periods: daily natural varroa mite fall on a sticky sheet covering the entire hive lower tray surface for seven consecutive days;
- Infestation level by *Nosema sp.* and *Acarapis woodi*;
- Bee population in the spring and at the end of the summer;
- Honey production;
- Colony survival during wintering;
- Oxalic and formic acids' residues in harvested honey.

[2] MINISTERE DE L'AGRICULTURE, DES PÊCHERIES ET DE L'ALIMENTATION. Détermination du taux d'acide oxalique et d'acide formique dans le miel et la cire d'abeille, MA. AcOx 1.0, Ministère de l'agriculture, des pêcheries et de l'alimentation, 2003.

[3] Nosema desease and control. Canadian Association of Professional Apiculturists. + Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, World Organisation for Animal Health.

Statistical analyses

The statistical analyses were completed with the help of the SAS software. For variables that do not respect a normal distribution (the varroa mite fall per day variables), the initial comparison of the four groups was completed through a Wilcoxon non-parametric analysis with the NPAR1WAY procedure. The other variables that respected normal distribution were submitted to a variance analysis with the GLM (generalised linear model) procedure.

Results and Discussion

Spring treatments are not effective in both tested pest management strategies. Figures 8.1 and 8.2 are showing that the natural varroa mite fall average increased from May to July (from 8.1 ± 3.0 to 17.8 ± 3.0 in IPM1 and from 5.7 ± 1.4 to 21.3 ± 6.6 in IPM2). Summer treatments with Mitewipe pads (2 pads/3x/3 days interval) have reduced natural varroa mite fall from 17.4 ± 3.0 varroa mites/day to 12.0 ± 1.8 varroa mites/day. Oxalic acid was ineffective during the same period: varroa mite fall went from 21.3 ± 6.6 varroa mites/day to 25.6 ± 6.8 varroa mites/day. Fall treatments are the most effective to reduce varroa mite populations in colonies. There is no significant difference in effectiveness between both pest management strategies between the months of September and November.

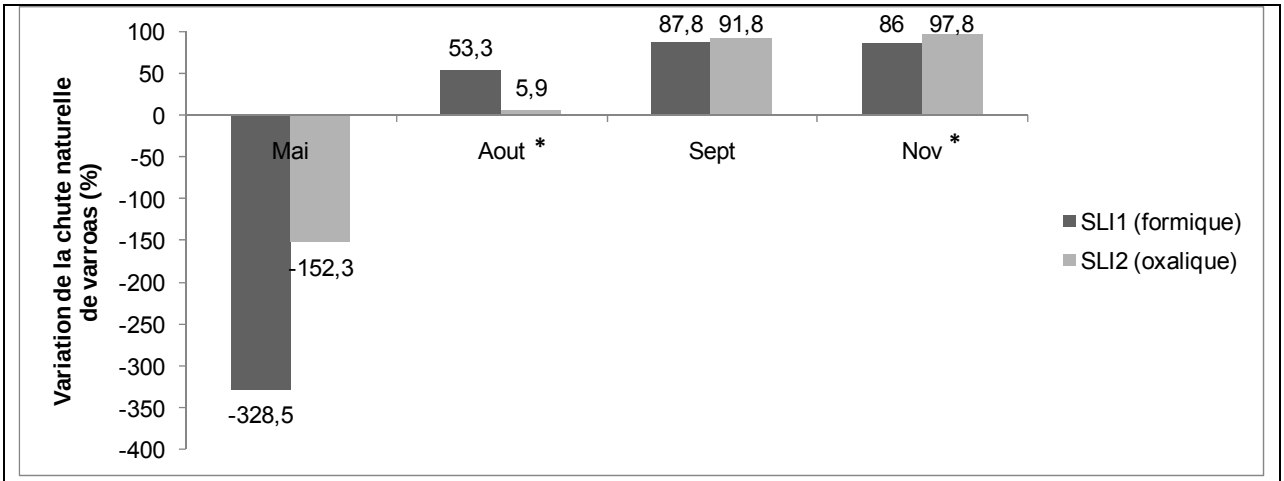


Figure 8.1. Effectiveness of treatments used within both integrated pest management strategies (IPM1: pest management strategy 1; IPM2: pest management strategy 2). Effectiveness is given by the reduction level of daily varroa mite fall measured before treatment and after treatment. An asterisk (*) indicates a significant difference (α<0.05).

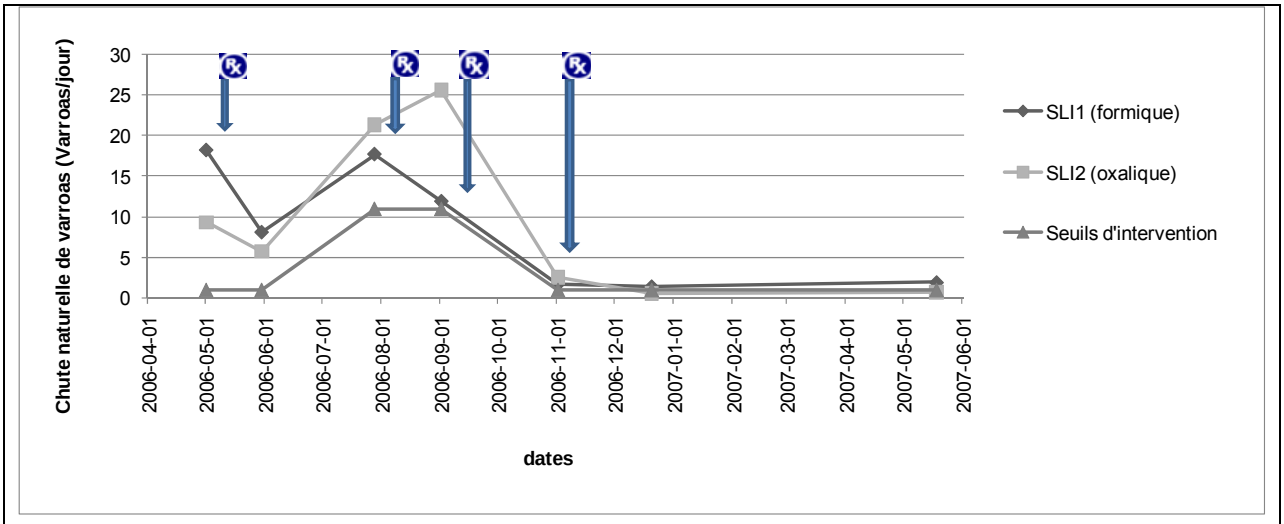


Figure 8.2. Evolution of the daily varroa mite fall for both integrated pest management strategies (IPM1: pest management strategy 1; IPM2: pest management strategy 2) during a beekeeping cycle. The various treatments' application periods are indicated with arrows.

Table 8.2 is indicating the other results achieved. Until November, 79% of IPM1 colonies survived, and 63% for IPM2. IPM2 required the renewal of three more queens than IPM1 in June. In the following year's spring, the survival rate of IPM1 colonies was higher than for IPM2.

From May to September, the number of bees and bee larvae (brood) are similar between hives of both pest management strategies. However, in November 2006, colonies were significantly stronger in IPM1 (bee frames: IPMS1=8.2 ± 0.2 > IPM2=5.0 ± 0.6; $\alpha<0.01$). A similar result was achieved in May 2007 (bee frames: IPM1=7.1 ± 0.4 > IPM2=3.4 ± 0.5; $\alpha<0.01$). We believe that these results are showing the impact of the high levels of varroasis measured in IPM2 colonies at the beginning of September (25.6 ± 27.3 varroa mites/day). The fall Apigard® treatment in the IPM2 group was effective in reducing varroa mites (average of 92% in natural varroa mite fall reduction); however, we suggest that the physiology of fall bees (that must spend the winter) was affected by the high varroasis levels measured in the fall.

Honey analyses from IPM2 colonies are indicating the presence of formic acid residues. (July honey = 330 ppm and 317 ppm; September honey = 127 ppm and 113 ppm).

Our analyses did not detect the presence of acariosis or nosema disease.

Table 8.2. Comparisons of both integrated pest management strategies. The different lowercase letters for a variable's data (lines) indicate a significant difference (α<0.5).

Variables	Integrated pest management strategy 1 (For the treatments' description, see Table 1)	Integrated pest management strategy 2 (For the treatments' description, see Table 1)
Number of active colonies		
- April 25, 2006	24 (100%)	24 (100%)
- May 31, 2006	23 (96%)	22 (92%)
- August 25, 2006	23 (96%)	21 (88%)
Note	4 queens changed in July	7 queens changed in July
- November 1, 2006.	19 (79%)	15 (63%)
- April 23, 2007	13 (54%)	9 (38%)
Hive strength (excluding dead bees)		
- April 25, 2006 (frames/bees µ±sd)	6.6±0.4[a]	7.3±0.4[a]
- May 31, 2006 (cells/brood µ±sd)	32016±941[a]	31935±1940[a]
- August 25, 2006 (cells/brood µ±sd)	19936±2393[a]	20957±2734[a]
- November 1, 2006 (frames/bees µ±sd)	8.2±0.2[a]	5.0±0.6[b]
- April 23, 2007 (frames/bees µ±sd)	7.1±0.4[a]	3.5±0.5[b]
Honey production (kg, µ±sd)		
May to September 2006	50.6±5.2[a]	49.5±8.0[a]
Residues in honey (2 samples/batch)		
- Oxalic acid		
July 20, 2006	21.5 and 21.4	21.0 and 21.7
September 9, 2006	21.2 and 21.5	21.2 and 21.6
- Formic acid		
July 20, 2006	330.0 and 316.9	0.0 and 0.0
September 9, 2006	113.0 and 127.0	0.0 and 0.0
Acariosis		
March 2007	Absent	Absent
Nosema disease		
March 2007	Absent	Absent

Conclusions

- Our results illustrate that a good control strategy must target an effective fall treatment in order not to make treatments in the spring.
- A summer treatment, between two honey flows with formic acid administered with Mitewipe pads (2 pads/3x/3 days interval) is sufficiently reducing varroasis levels until the application of a fall treatment; however, beekeepers must expect to have formic acid residues in their honey harvest.

- The pest management strategy using formic acid (IPM1) has allowed for good colony development during the season. This development is superior to the strategy 1 colonies after wintering in April.
- Spring treatments made in both pest control strategies (formic acid or oxalic acid) are not preventing the growth of varroasis populations during the summer.
- Acariosis does not seem to be influenced by treatments.

9. Evaluation of different treatments against varroasis on nuclei in the fall

Goal

- To verify the effectiveness of organic anti-varroa mite treatments on nuclei installed at the end of the summer.

Material and Method

The nuclei used were prepared in August of 2005. In the beginning, each nucleus has two brood frames, a food frame, an empty frame, a young queen from a grafting on selected hives of the Centre de recherche en sciences animales de Deschambault and this mini-colony is filled with bees. In September, these four-frame nuclei were placed 2 by 2 on a standard hive base with an anti-varroa mite tray separated in the middle by a 1/2'' x 1'' wood board (pine).

The tested control methods used thymol and formic/oxalic organic acids. The group 1 nuclei did not receive treatment (control); the group 2 nuclei were treated with 65% formic acid (application of a Mitewipe soaked with 35 ml of 65% formic acid per nucleus every three days and repeated three times); the group 3 nuclei received a thymol application in Apigard® form (thymol in gel, 12.5 g per nucleus repeated once after 14 days); the group 4 nuclei received a thymol application in Thymovar® form (thymol in plates, ½ plate per nucleus repeated once after 14 days); the group 5 nuclei received an oxalic acid application (through dripping, 5 ml per bee frame of a 40 g/L oxalic acid solution in 1:1 saccharose syrup). Nuclei placed in pairs were randomly distributed in the different experimental groups of the protocol illustrated in Table 9.1. Treatments started on October 14 for all nuclei groups, except for the one in which colonies were treated with oxalic acid that started on November 4. This delay was necessary, since oxalic acid is only effective when there is little or no more brood in colonies.

Table 9.1. Research protocol and description of experimental groups and different anti-varroa mite treatments tested.

Group 1	Group 2	Group 3	Group 4	Group 5
Without treatment	Mitewipe 1 Mitewipe / nucleus (35 ml of 65% formic acid) Repeated 3 times every 3 days	Apiguard 12.5 g / nucleus Repeated once after 14 days	Thymovar ½ plate / nucleus Repeated once after 14 days	Oxalic acid 5 ml / bee frame (40g/L of oxalic acid in 1:1 syrup)
N=8 nuclei (4 pairs)	N=8 nuclei (4 pairs)	N=8 nuclei (4 pairs)	N=8 nuclei (4 pairs)	N=8 nuclei (4 pairs)

The dependent variables measured are:

- Evolution of varroasis from September 2005 to May 2006: the parasitism level by *Varroa destructor* is assessed based on the natural fall method. A sticky sheet covering a third of the lower surface was placed under the frames of a colony for a minimum of five days. All these data are presented in daily fall of varroa mites.
- Evolution of the colonies' strength from September 2005 to May 2006: the colonies' strength was evaluated by the weight or the number of frames occupied by bees in the brood chamber (1 to 4).

Results and Discussion

Table 9.2 is indicating results achieved. All colonies survived following the different tested treatments until the beginning of the wintering period in a repository (December 21, 2005). However, after being taken out after winter (April 2006), half of colonies that received the oxalic acid treatment and the Thymovar® treatment were dead. Two nuclei were dead in the control group and the Mitewipe group and only one colony was dead in the Apigard® group. Just before being put in the repository for wintering, colonies had similar weight. When they were taken out of wintering, nuclei of all experimental groups had between 2 and 3 bee frames, except for the nuclei group treated with Thymovar®

(non-significant difference $\alpha>0.05$). After the first month of spring, we can see two statistical groupings based on the colonies' strength measured with the number of brood on frames: colonies of the Mitewipe, oxalic acid, control, and Apigard® groups and the colonies of the oxalic acid, control, Apigard®, and Thymovar® groups. Of these two groupings, colonies of the Mitewipe group presented nuclei with the strongest spring resumption, while colonies of the Thymovar® group has the weakest resumption (10,936 brood cells, as opposed to 6,292 brood cells, $\alpha<0.05$).

Table 9.2. Results achieved during the different fall treatments on young bee nuclei.

	Group 1 Control Without treatment	**Group 2** Experimental Mitewipe	**Group 3** Experimental Apiguard®	**Group 4** Experimental Thymovar®	**Group 5** Experimental Oxalic acid
Number of active colonies					
- Sept. 9, 2005	8	8	8	8	8
- Nov. 11, 2005	8	8	8	8	8
- Dec. 21, 2005	8	8	8	8	8
- April 19, 2006	6	6	7	4	4
- May 5, 2006	6	6	7	4	4
Hive strength (excluding dead bees)					
- Nov. 17, 2005 (kg µ±sd)	36.8 ± 2.8[a]	35.33 ± 2.4[a]	36.5 ± 0.5[a]	39.2 ± 0.1[a]	34.8 ± 3.7[a]
- April 19, 2006 (kg µ±sd)	29.5 ± 2.8[a]	27.4 ± 2.6[a]	27.4 ± 1.0[a]	30.5 ± 0.6[a]	26.8 ± 4.6[a]
- April 19, 2006 (bee frames µ±sd)	2.3 ± 0.2[a]	3.0 ± 0.3[a]	2.4 ± 0.3[a]	1.8 ± 0.1[a]	2.1 ± 0.4[a]
- May 5, 2006 (cells/brood µ±sd)	9967 ± 1478[ab]	10936 ± 1591[a]	9596 ± 1138[ab]	6292 ± 1721[b]	10588 ± 1411[ab]
Varroasis					
- From Oct. 11 to Dec. 21					
Total varroa mite fall	12.9 ± 2.8[c]	171.5 ± 38.0[ab]	57.1 ± 15.7[bc]	84.9 ± 52.0[bc]	269.1 ± 84.2[a]
Treatment effectiveness (reduction %)	20.4 ± 11.1[ab]	41.2 ± 10.5[ab]	14.2 ± 4.6[a]	3.4 ± 8.5[b]	21.2 ± 12.3[ab]
- May 1, 2006					
Natural varroa mite fall/day	0.4 ± 0.2[a]	0.1 ± 0.1[a]	0.5 ± 0.2[a]	0.6 ± 0.1[a]	0.3 ± 0.1[a]

The total varroa mite fall between October 11 and December 21 is significantly different between groups. We can see in Figure 9.1 that there was an increase in varroa mite fall in colonies treated with formic acid (Mitewipe from October 14 to November 11) and colonies treated with oxalic acid through dripping (November 11 to December 5). This is an indication of the acaricidal effect of these two treatments with acids. However, the evolution of the daily fall in both groups treated with thymol is similar and close to that of the control group, that is not

showing any fluctuations. The Table 9.2 data are showing that two treatments distinguish themselves on a statistical level: the formic acid and oxalic acid treatments. The control group has the lowest fall, while the two other treatments with thymol have intermediate fall levels. When comparing the daily fall reduction level between the period beginning before treatments and ending when taken out of wintering in April, we can see that the reduction level was higher in colonies of the colony group treated with formic acid (Mitewipe); however, this value is not statistically different than the control, oxalic acid et Apigard® groups. The group with the lowest reduction level is the one treated with Thymovar®. The measured daily natural falls in the spring are low for all groups.

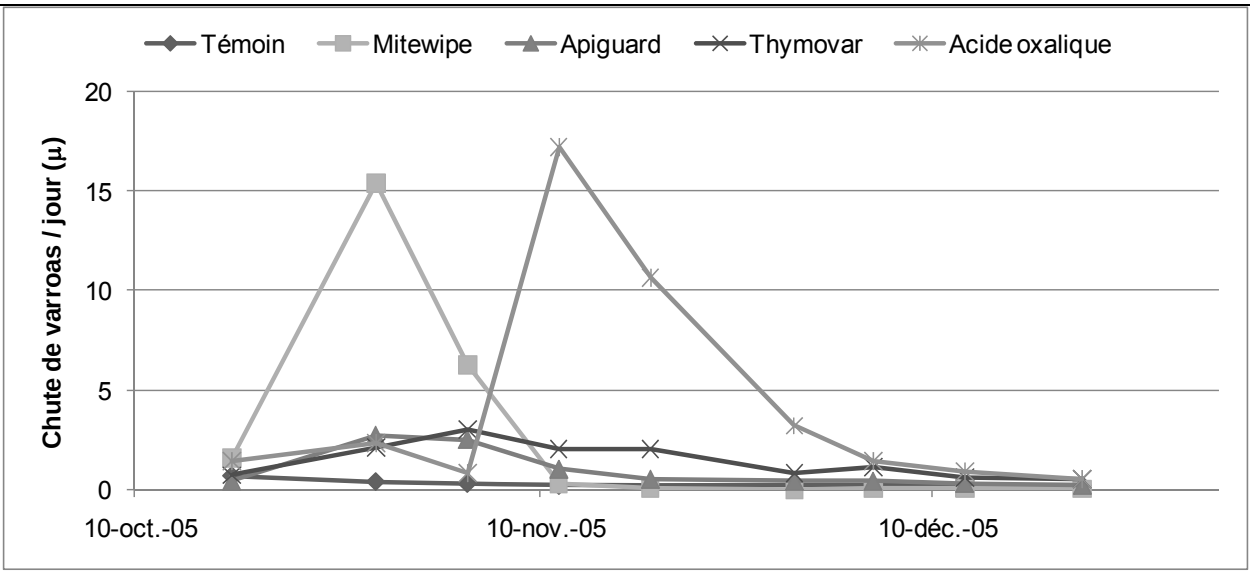


Figure 9.1. Evolution of the daily varroa mite fall between October 14 and December 21, 2005. The Mitewipe, Apigard, and Thymovar treatments started on October 14, while the oxalic acid treatment started on November 4.

Conclusions

- It is possible to make an effective treatment against varroasis in nuclei in the fall.
- Fall treatments with oxalic acid through dripping and formic acid (Mitewipe) have an acaricidal effect on varroa mites.
- Colony survival after wintering is lower for colonies that received the oxalic acid treatment.
- Colonies treated with formic acid (Mitewipe) survived wintering well.
- Colonies treated with formic acid (Mitewipe) were stronger after taken out of wintering and their spring resumption was higher than the other groups.