EVALUATION OF MITEGONE FORMIC ACID TREATMENT AS AN EFFECTIVE CONTROL OF VARROA AND TRACHEA MITES AND VARIOUS APPLICATIONS OF MITEGONE PADS AS SMALL HIVE BEETLE REPELLENT IN A SUBTROPICAL CLIMATE – PHASE 1

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[2] Fictitious names of beekeepers who face mites resistant to all known pesticides and a large population of beetles. They provided all material and labour in the test in an undisclosed location.

Note: EPA ruling: There is no law on the US federal books to prevent a beekeeper from using unregulated substance (formic acid) in his own hives, however the state and local policies may vary widely including persecution and fines.

ABSTRACT

VARROA MITE

MiteGone's method of dispensing 65% concentrated formic acid was applied in the first week of November to evaluate the effectivity of standard semi-annual treatments in a subtropical climate using common configuration of hives in this region (deep and shallow).

The standard treatment showed on the first day of application an efficacy multiple of 7.81 to 8.23 which translates to 80-85% efficacy (Ruzicka et al. Testing Manual 2004).

Note: Formic acid applied using the MiteGone method and dispensers, besides having the 75-95% efficacy in the primary treatment period of 21-35 days, makes surviving mites sick and they keep dying or not reproducing for a long time after that in the extended treatment period which really lasts until the next semi-annual treatment in spring (tentatively mid-April).

Only natural drop before the treatment in April will confirm the success of the treatment in the fall.

SMALL HIVE BEETLE

All the standard MiteGone treatments and various installations of pads with the purpose of repelling SHB has been successful of repelling even high infestations of SHB (hundreds on the top and bottom) within 3-5 days. We do not know at present if it happens any faster (we did not check) or if the beetle got killed or just left. The fact is it went away and did not return for the presently available observation of 12 weeks at Feb 20, 2008, the time of the writing of this paper.

The advantages, effectiveness, methods, and reasons for both subjects are discussed.

INTRODUCTION

The parasitic mite, *Varroa destructor* Anderson & Trueman, is arguably the single greatest challenge to successful beekeeping in the United States. Without intervention, honey bee (*Apis mellifera* L.) colonies typically die within two years after initial varroa infestation (DeJong et al. 1982a). For over 15 years, effective varroa control strategies in the US have centered on the use of conventional pesticides. However, the consistent and exclusive use of these products to control varroa mites have led to additional problems for beekeepers, the most serious being the development of varroa populations that are resistant to fluvalinate (Apistan®, Wellmark, Bensenville, IL), coumaphos (Checkmite+®, Bayer, Shawnee Mission, KS), and unregistered Amitraz (Baxter et al. 1998; Elzen et al. 1998; Elzen and Westervelt 2002), and the potential contamination of hive products with the pesticide residues (Wallner 1999).

The small hive beetle in conjunction with varroa makes beekeeping in the southern states extremely difficult and while present control with coumaphos keeps it in check, the SHB eventually will become resistant to it and a different approach will be required.

Other alternative approaches to varroa and SHB control are the use of natural substances having the ability to control mites. These substances may offer beekeepers practical, yet effective means of parasite control, and are therefore strong candidates for incorporation into varroa IPM and pesticide resistance management programs. A considerable amount of research on the tracheal mite (*Apcarapis woodi* (Rennie)) and varroa mite control with organic acids (formic, lactic, and oxalic acid) and essential oils (thymol, eucalyptol, etc.) has been conducted in Europe, the US, and elsewhere (Imdorf et al. 1996; Fries 1997; Thomas 1997; Nanetti et al. 2003). Exploratory tests on the control and repelling of SHB with formic acid and MiteGone application have been conducted in a subtropical condition since 1998 without publication (Ruzicka et al.).

In the late 1990s, a formic acid gel formulation (Apicure®) was developed for use in the US for the control of tracheal mites and suppression of varroa mites (Feldlaufer et al. 1997). Dispite reasonable efficacy against both mites, Apicure® was removed from the market shortly after its introduction due to packaging problems. However, various formic acid pads and dispensers are available and used routinely by beekeepers in the US and other countries (Nasr 1996; Calderone 1999; Fries 1997; Ruzicka 1994).

MiteGone dispenser and method has been available in the USA since 2001 and as a dry dispenser it is not subject to EPA registration. The efficacy was tested in USA (Strangellini, Reinbold 2004, 2005) in Canada (Melapopulos, Gates, Ruzicka 2001), Spain, and the Canary Islands (Felitas y Pauleo). Theirs and others' papers on the subject are available at www.mitegone.com in Scientific Evidence.

The use of formic acid by the beekeeper on his own hives is considered legal by federal authority, and many beekeepers use the short blast methods of butcher pads, blue towels, and other methods, but will not admit it publicly being uncertain about local policies.

Recently a prefilled pad product MITEAWAYII was registered in the USA. It is a replica of the old German patent #3427330 known as Kramer Plate and inherited all the problems the Kramer had from brood and bee mortality to absconding, low efficacy, and complete failure in subtropic conditions in tests in 2004 (Elzen, Patti J., David Westervelt and Raymond Lucas, 2004. *Formic acid treatment for control of Varroa destructor (Mesostigmata: Varroidae) and safety to Apis mellifera (Hymenoptera: Apiadae) under southern United States conditions.* Journal of Economic Entomology, 97: 1509-1512. Mite-Away pads have a low efficacy of 51-56% (Caledrone 1999, Caledrone and Nasr). (Nasr 1996)). These and

other failures are due to misinterpretation of instructions, misapplication of treatments and plain inexperience with the applicators and high concentrations of formic acid.

Purposely spread rumours of the danger of formic acid, legality of its use, and an entire lack of real research, scientifically and statistically correct trials led to more rumours:

- Formic acid is not suitable for treatment in subtropical climates
- Formic acid attracts/repels SHB into hives
- There are no suitable target times for effective treatment in subtropical climates
- Danger of contamination of honey by formic acid

We leave alone the question of legality of its use. Where is the common sense when the state official (North Carolina in Sacramento workshop) considers the use of liquid formic acid illegal but the use of the same acid impregnated in pulp for 10 times the cost to the beekeeper is legal. You can register a pre filled product? No one wants to register a commonly sold unregulated substance like formic acid (Bee Culture, March 2004, Volume 132, Issue 3, Page 37)

It is up to the US bee industry and regulatory organizations to exempt or officially declare the use of liquid acid by beekeepers to be legal in the US to stop the persecution of beekeepers.

In this study we evaluated : The efficacy of the MiteGone method and dispenser of 65% formic acid. Verified the target treatment times of the year. Dosage and evaporation rates to control varroa and trachea mites levels. The effect of standard treatment and various repellent installation of MiteGone pads on attracting, repelling, or deterring SMALL HIVE BEETLE from the hives. The contamination of honey and acceptance of formic acid treatment in organic production.

MATERIALS & METHODS

Colonies: 32 colonies were selected out of 80 in the out bee yard located in a typical subtropical climate on level land with large oak trees spaced widely providing sunny and shady coverage on all hives in the test in rotation due to the sun's movement. The grass was kept low by the presence of cows grazing on the same property.

All colonies were of typical arrangement for the gulf states region: all were on single bottoms with deep brood chambers containing nine standard deep frames, separated with Q excluder from shallow honey super with eight frames on metal spacers. Honey was extracted before test and shallow supers installed were empty. There was still a sufficient supply of nectar and pollen so no feeding of syrup or other supplements were required.

TABLE 1. PRIOR TO TEST: HIVES EVALUATION – GROUP ASIGMENT AND INITIAL EFFICACY OF TREATMENTS

Hive Condition				Bees in Bot	Bees in Top	Weight	Beetle Presence Bot	Beetle Presence Top	Time of Day	Natural Drop Bill Tot.	Natural Drop 24 Hr day	Group Assignment	Hive No.	Acid Drop 24 Hrs Bill	Acid Drop 24 Hrs Steve	Initial Efficacy Bill	Initial Efficacy Steve
No	Q	Age Queen	Brood Frames	Dep 9-fr	Shal 8-fr												
1	Q	1 year	9	9	8	50	0		11:00 am	28	9.3	A	1	79	89	9	9.5
2	Q	1-2	8	9	8	40	0			49	16.3	D	2	-	-	-	-
3	Q	1	8	9	8	45	0			18	6.0	D	3	-	-	-	-
4	Q	1	6	9	8	35	0			37	12.3	D	4	-	-	-	-
5	Q	1	7	9	8	40	1 – 0			30	10.0	B	5	17	19	2	2
6	Q	1 – vishis	7	9	8	50	0			20	6.6	A	6	11	11	2	2
7	Q	1 – 2	7	9	8	50	0			25	8.3	B	7	75	68	9	8.19
8	Q	1 – 2	7	9	8	60	6			10	3.3	D	8	-	-	-	-
9	Q	1 –	7	9	8	50	0			13	4.3	A	9	14	18	3.5	4.18
10	Q	1 –	6	9	7	60	0			13	4.3	B	10	19	26	4	6
11	Q	1 –	7	9	8	35	5		12:30 pm	26	8.6	B	11	119	143	14	16.62
12	Q	2 –	5	6	8	50	1 – 0			16	5.3	D	12	-	-	-	-
13	Q	1	8	9	8	40	0			97	32.0	A	13	104	112	3.2	3.5
14	Q	1	7	9	8	50	0			30	10.0	A	14	91	128	9	12.8
15	Q	1	6	9	6	50	0		1:00 pm	4	1.3	A	15	7	6	7	4.6
16	Q	1	7	9	8	60	1			8	2.6	B	16	13	17	5	6.5
17	Q	1	9	9	8	50	0			24	8.0	D	17	-	-	-	-
18	Q	New	9	9	8	50	0 – 8			27	9.0	D	18	-	-	-	-
19	Q	1	6 ½	9	8	50	0			7	2.3	B	19	33	38	15	16.56
20	Q	1	9	9	8	50	2			11	9.6	A	20	87	127	9	13.22
21	Q	1	7	9	8	50	1			34	11.3	D	21	-	-	-	-
22	Q	1	8	9	8	50	0			8	2.8	A	22	44	52	18.2	20
23	Q	New	6	8	6	50	0			9	3.0	B	23	3	3	1	1
24	Q	1	7 ½	9	8	45	0		2:00 pm	15	5.0	B	24	25	34	5	6.8
25	Q	1	6	9	8	50	1 – 2		1:00 pm	44	14.2	C	25	Average Efficacy			
26	Q	1	6	9	8	45	1 – 2		1:00 pm	12	4.0	C	26	-	-	-	-
27	Q	1	7	9	8	50	1 – 2			12	4.0	C	27	Group	A	7.80	8.73
28	Q	1	6	9	6	45	1 – 2			3	1.0	C	28	-	B	6.90	7.95
29	Q	1	8	9	8	50	0			6	2.0	C	29	-	-	-	-
30	Q	1	7	9	8	50	0			34	11.3	C	30	-	-	-	-
31	Q	1	6	9	6	40	0			34	11.3	C	31	-	-	-	-
32	Q	1	6	9	8	50	3 – 4		2:30 pm	9	3.0	C	32	-	-	-	-

Each selected colony had a laying queen, 5-9 (average 7) frames of brood in various stages. Bees were covering nine frames in deep brood chambers and 7-8 frames in the top shallow super. The average weight of colonies was 40-50lbs. A random collection of ten flying forager bees from each colony in a 50/50 alcohol solution in one jar was sent to a reputable lab for slicing. No trachea mites were found in the first 50 bees, resulting in non-detectable level (Ruzicka et al. 2004 Manual of Treatments and Testing). All hives were checked for the presence of SHB. In most hives there were no beetles, 1-8 were in a few hives, in 5 hives wooden veneer strips 2x8”were found on the bottom board, underneath were 30-50 beetles hiding. Within a mile in the home yard and other out yard there were hives with a high level of SHB infestation, proving that the area of test did not lack a population of SHB.

The hive examination and drop test application of boards were done between 11am and 2:30pm in sunny, dry weather ensuring equal judgment of colonies by the lead beekeeper. The writer strictly observed and recorded the data. See Table 1.

Later, in the fourth week of testing after failing to attract the beetle into the installed traps of Group D, another seven hives were selected as group E in the main yard on a similar strength but high infestation of the beetle, and more similar hives were added later to test various repellent methods.

WEATHER was relatively dry with sunny periods and mild temperatures typical for the region in the beginning of November. We measured inside and outside temperatures and humidity in three randomly selected hives for the first five days of the treatment period, in place where pads are installed, as follows:

Table 2. Inside and Outside Temperature in °F, Relative Humidity in %																
Date	Hive #1				Hive #19				Hive #24				Outside Yard			
Time:	Morning		Afternoon		Morning		Afternoon		Morning		Afternoon		Morning		Afternoon	
	T	H	T	H	T	H	T	H	T	H	T	H	T	H	T	H
Nov 2	/		90*	68	/	/	100*	68	/	/	/	/			86	52
Nov 3	70	68	84	51	70	76	88	52	74	76	84	51	65	62	77	45
Nov 4	70	73	84	55	74	72	83	52	74	73	82	54	65	68	80	48
Nov 5	71	64	85	50	66	63	84	49	65	64	79	50	62	58	77	37
Nov 6	66	70	87	53	65	69	87	56	64	69	84	49	61	65	81	32
Avg.	69.2	68.7	86	55.4	68.7	70	88.4	55.4	69.2	70.5	82.2	51	63.2	63.2	80.2	42.8

**High afternoon temperature on hives 1 and 17 are the result of full sun exposure to the measured side of the hive. November 2 was out of the norm, a hot dry day with full sun. The rest of the days were more to the normal, average kind of weather for the first week of November.*

Treatments: All colonies were identified as being infested with varroa mites by using the 3-5 day natural drop prorated to 24 hours using 16x12 inch plastic sticky boards and raised metal screens technic (Ruzicka et al. Treatments and Testing Handbook 2004) and split into four groups of eight hives each, according to similar sized colonies and the level of infestation for groups 'A', 'B', and 'D' being equal. Having only twenty-four sets of boards and screens, the control group 'C – controls' was selected on the same strength, weight, and bee population but drop tests were performed three days later, giving the control group the advantage of having an average of 30% lower mite population of 6.35, while the treatments of groups A, B, and D have 9.46 mites per 24 hour prorated natural drop before treatment.

All board counts were performed by two people to ensure the correctness of counts and to train the lead beekeeper in the technique to ensure continuity and accuracy of data collected at the end of the secondary treatment period in April of 2008. A comparison of counts showed that Steve's, while being generally higher, are well with in the statistical limit, and we will attempt to have the final counts recounted by an independent lab on groups A and C. Counts will be done on groups B and D, their results are only of supportive roles. See discussion of each group.

APPLICATION OF TREATMENTS A, B, and D were done on November 7, 2001, being a first day of treatment drop tests for efficacy were collected on that day, providing 24 hour acid induced drop. When compared with natural drop it provides an indication of how well the treatment works on the first day (Ruzicka et al. Manual of Treatments 2004).

MITE MORTALITY DATA: The count on group A – 7.8 (Bill) and 8.73 (Steve) means that the acid application dropped that many more mites than prorated natural drop before treatment and with relatively low infestation this number is considered to be very good. 85-90% efficacy.

END OF PRIMARY TREATMENT: No mite counts were taken on day 35, which is deemed to be the end of primary treatment (21-35 day period) as mites continue dying from exposure to formic acid for a long time, and in many cases drops at the end of the primary treatment may be higher than drops before treatment.

The only true measure is the natural drop level before the next semi-annual treatment planned for April 2008 (Ruzicka et al. Manual of Treatments 2004, 2008).

TIMING OF THE TREATMENT: It is important to treat before the mite can cause damage to winter brood. Treatment must start and primary portions should be finished before the queen reduces the laying to 2-4 frames of winter bees. One of the purposes of this test is to verify that we found the target treatment time for a subtropic climate.

TREATMENT GROUP A: 8 hives were treated with 360 grams of 65% formic acid dispensed by three half-pads having a constant evaporating rate under 75°F and 55% relative humidity of 6 grams per day, delivering 18 grams total per 24 hour period in low continuous dose, not causing any distress or damage to the colonies.
The answers sought are:

- Is the dosage sufficient to provide a minimum
- of 21 days primary treatment?
  (with slight variable temperature and humidity inside the hive the evaporation rate will vary)
- Is the dosage sufficient to provide satisfactory
- primary efficacy?
- Will this application attract or deter the small hive beetle?

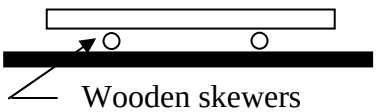
TREATMENT GROUP B: 8 hives were treated by 240 grams of 65% formic acid dispensed by 4 quarter pads having the same evaporation rate as half pads under the standard condition, delivering 24 grams of acid in a 24 hour period. The answers sought are:

- Will a 30% increase in the daily dosage provide better initial efficacy?
- How long will the ¼ pad last in a real colony? Will it provide the correct evaporation rate?
- How short will the primary treatment be?
- What will the mite levels and final result in April be?
- Will this application attract or deter SHB?

CONTROL GROUP C: Received no treatment and is monitored for the presence of SHB.

- The purpose is to find out what level of varroa and beetle untreated colonies will have in April.

TREATMENT GROUP D: Received treatment by 240 grams of formic acid dispensed by an uncut pad installed on the bottom board over 1/16 inch thick skewers. This installation was found in the preliminary exploratory test as having after 8 weeks dead SHB underneath the pad. The answers sought:



- Will this trap attract or repel the beetle? Will it kill it? How long will the evaporation be and at what rate?
- Will this application have any effect as treatment for varroa?

TREATMENT GROUP E: Was added in week four after Group D did not have any beetles under the pads at any time. Seven colonies in the main yard having high infestation of beetles, (literally hundreds on the top and bottom), were treated with the same arrangement as Group D. The check was made in five days and most of the beetles were gone.

TREATMENT GROUPS F TO Z: are treatments described below, and applied to various sizes of colonies to prove or disprove repellent installation length of treatment and effectiveness in different times and weather conditions of the year. Which of three applications is the most desirable under subtropical conditions or conditions in other effected states?

While Phase I will report on the already found answers, full or partial, the April 2008 results will be published in Phase II.

THEORY AND KEY TO SUCCESS:
The theory behind these observations is that the acid repels the beetle either because the acid smells so bad so that the beetle leaves or because the acid somehow distorts the pheromones that attract the beetles to the hive especially to queen-less hives or hives in distress.

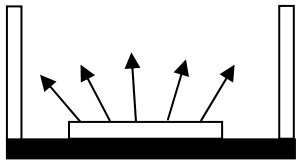
Harsh application of formic acid will cause distress and attract beetles to the hives.
(Harsh and high concentrations makes bees kill their queens, causing distress (Clark et al.).)

MiteGone uses a low dose continuous release method which, with a low 65% concentration, does not disturb hives but repels the beetle.

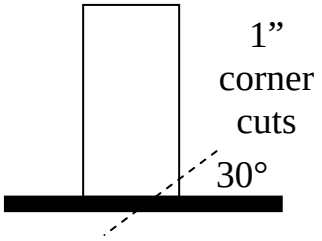
STANDARD MITEGONE TREATMENT FOR MITES: three half-pads in the top box will not disturb hives. It will repel the beetle for a minimum of five to six weeks.

REPELLENT METHODS: We know that one full MiteGone pad will hold 240-250 grams of acid.

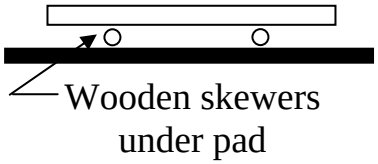
- Laying the pad flat on the bottom board or placed in a tray under a screen bottom board will emit 2-4 grams of acid per day through the perforations. It will last 2-3 months, increase efficacy of screened bottom boards with collecting trays, and repel the beetle.



- A pad placed vertically between the comb and the wall of the hive body standing on the bottom board with a one inch corner cut or mid notch will emit 1.5-2 grams a day and last up to 5 months.



- Beetle trap: lay uncut pad on bottom board, supported by skewers. Repels heavy infestation but lasts six to eight weeks.



Answer sought: Which of the methods is most desirable and will work best in a subtropical climate?

OBSERVATIONS & RESULTS

Day 14 – November 22, 2007 TREATMENT OF MITES

Group B 4 quarter pads are dry; proving sufficient evaporation rate took place.

Group A 3 half-pads are not yet dry; proving intended evaporation of 21 – 35 days will be achieved.

Group D Full pads on 1/16 thick skives. All pads still contain acid. Approximately half of the perforated holes on two pads had been propolised on the top. Four pads propolised to bottom board to close the 1/16 space between pads and bottom board. Four pads have not been propolised.

Clean Pad on Skewers

Propolised Pad on Skewers

EFFECT ON SMALL HIVE BEETLE: Area has plenty of beetles. We found a few beetle larvae in the debris on the drop test boards after 3 days and plenty of beetles hiding under test boards. In Groups A and B were pads installed between the frame and wall of shallow super with 8 frames on metal spacers. Narrow space for beetles to hide between the pad and wall of super perfect for beetle to hide, did not have any beetles in the area of pads at all.

Group D with the length of pad over 1/16 thick skewers purposely creating a hiding place for beetle. No beetles were found under the pads.
No beetles ere found in a substantial number under the top or on bottoms covers of all treatment groups including controls. Confirming that strong colonies will keep the beetle out and MiteGone formic acid application on strong colonies does not attract the beetle; on the contrary, it may deter it.

Day 28 – December 5, 2007 TREATMENT OF HIVES – EVAPORATION OF ACID
Group A 3 half pads all fully evaporated residues 10 – 14g, empty pad 8g. **This confirms the targeted evaporation period of 21 – 35 days was reached as on day 14 pads were half full.**

Group B 4 quarter pads fully evaporated on day 14.

Group C Control group - no pads or treatment. No visual damage by mites.

EFFECT ON SMALL HIVE BEETLE: Hive #20 in Group A has 4 – 5 beetles at the top cover. All other hives, including controls, did not have beetles on top or bottom.

Group D No beetle found under pads installed on bottom. Some acid remains. Half of pads are partly one fully propolised.
Installation of pads on skewers obviously will not attract the beetle to hide under pads and get effected by the acid. What will happen in infested hives?

REPELLING THE BEETLE FROM HEAVILY INFESTED HIVES:

Group E was established as 7 hives were found in a home yard with high infestation of the beetle (literally hundreds under the top cover and on the bottom board) Full pads were installed on 1/6 skewers, as in group D. Upon examination 5 days later just a few beetles were found hiding in combs of honey supers.

Conclusion: Small low-dose continuous release of 65% formic acid using MiteGone pads will repel a high infestation of beetles out of the hives.

Day 35 – December 12, 2007 – 5 weeks END OF PRIMARY TREATMENT
Last observation jointly preformed by Bill and Steve (Bill is leaving the test site and returning to Canada). All hives were checked for laying queen and all were found queen right and strong.

TIMING OF PRIMARY VARROA TREATMENT.
Count of brood and bees was done on Group A standard treatment and C controls with brood combs in the brood chamber. One to seven combs of bees were in shallow supers.
The primary treatment target period in 2007 for this area November 7 through December 12, three to five weeks was correct.
In the visual observation no varroa damage to the bees was found.

Group B – The quarter pads were removed and disposed of as they are not reusable.

Group A – The half pads were left in for reuse in April.

TABLE 3 – HIVE EXAMINATION AND BEETLE PRESENCE ON 35TH DAY AND OF PRIMARY TREATMENT PERIOD – BROOD & BEES COUNT ON A & C GROUPS ONLY

Hive Condition Q Right				Bees in		Beetle Presence		Pads – Dry or % of Acid Remaining	Pads Condition and Remarks
				Bot	Top				
Group	No.	Q	Brood Frames	Dep 9-fr	Shal 8-fr	At top	C Bottom		
A	1	Q	4	9	3	No	No	Dry	
D	2	Q	-	-	-	No	No	Dry	
D	3	Q	-	-	-	No	No	20%	
D	4	Q	-	-	-	No	1	5%	
B	5	Q	-	-	-	No	No	Dry	
A	6	Q	2	9	7	1	No	Dry	
B	7	Q	-	-	-	No	2	Dry	Chewed up
D	8	Q	-	-	-	8	No	25%	All propolised
A	9	Q	5	9	1	No	2	Dry	
B	10	Q	-	-	-	1	No	-	Removed
B	11	Q	-	-	-	8*	1	-	*Some in comb
D	12	Q	-	-	-	1	No	20%	Clean
A	13	Q	3	9	4	No	No	Dry	
A	14	Q	4	9	6	No	No	Dry	
A	15	Q	3	8	7	No	4	Dry	
B	16	Q	-	-	-	No	No	-	Removed
D	17	Q	-	-	-	2	No	Dry	Clean
D	18	Q	-	-	-	10*	No	30%	*No bees in top super
			-	-					
B	19	Q	-	-	-	No	No	-	Removed
A	20	Q	3	9	7	5 bs	3	Dry	
D	21	Q	-	-	-	20	No	10%	Propolised front
A	22	Q	3	9	4	No	No	Dry	
B	23	Q	-	-	-	6	2	-	Removed
B	24	Q	-	-	-	1	No	-	Removed
C	25	Q	2	9	6	7	No		Some– in comb
C	26	Q	2	9	6	No	1		
C	27	Q	3	9	4	No	No		
C	28	Q	4	9	6	2	No		
C	29	Q	6	9	8	No	No		Extra Strong
C	30	Q	3	9	7	No	1		
C	31	Q	3	9	5	1	No		
C	32	Q	2	9	7	3	No		

BEETLE OBSERVATION:
All hives were examined for the presence of beetles (See Table 3).
At the bottom: Group A – 2 , B – 3, C – 2, D – 1,hive , 23 hives 0.
At the top: Group A – 2 hives, B – 4, C – 4, D – 5, hives ,17 hives 0.

Group A had the least beetles with fifteen in total on three hives.
Group D had the most beetles with forty-one in total but in six hives.
Group C had the same as A with fifteen in total but in six hives.
While there are some high numbers in Group D these are explainable. For instance, D-18 had ten beetles in an empty super with no bees, and D-21 with twenty and D-8 with eight had pads fully or partly propolised. Group A seems to have the best result: the numbers of the beetle populations are too low to have any effect.
The only conclusion is that strong hives will keep the SHB out and that MiteGone treatment does not attract the beetle to the hives.

January 7, 2008: Steve finds another two hives in the main yard with substantial beetle population and varroa damage, and apply three half pads standard treatment to those hives, establishing **Group F**.

Day 63 – January 12, 2008 – 9 weeks – Secondary Treatment Period: as agreed, Steve makes periodical observations:
Groups A, B, C, and D all strong with no signs of varroa damage or beetle presence.
Group E established six weeks ago to repel beetle, six hives have no beetle in tops and bottoms. Few were found in the comb of super (less than ten). One hive, which is on the weak side and the pad was fully propolised in the fourth week after application, now has thirty beetles in total.
Group F beetles left the colonies.

Day 77– January 24, 2008 – 11 weeks – Secondary Treatment Period
Groups A, B, C, and D all strong with no signs of varroa damage or substantial beetle presence.
Group E only a few beetles returned (less than 20), but all hives display visible varroa damage. Steve decided to apply two flash treatments of 25cc of 65% formic acid in seven days intervals dropping 200-250 mites each time but killing one queen.
Group F in three weeks has no beetles and no visible damage by varroa.

Day 98 – February 21, 2008 – 14 weeks – Secondary Treatment Period: last observation for Phase I of this paper, closing for publication.
Groups A, B, C, and D all strong with no sign of varroa damage or substantial presence of the beetle.
Group E twelve weeks from treatment has no substantial beetle population. Some were hiding in the top super comb (less than 20). Has no varroa damage to the bees.
Group F now in seven weeks of the three half-pad standard treatment . The population increased, there was no visual varroa damage, and no beetles. Pads are now dry and left for reuse in April.
Note: Group F can only be considered to be exploratory, having two hives. But over twelve hives in groups of two and three displayed the same results in exploratory tests in the last five years and led to discovery of Mitegone pads and treatment as repellent for SHB.

SUMMARY OF RESULTS

MAIN QUESTIONS ANSWERED:

- Yes, the standard MiteGone fall treatment will work, provide good control of varroa, and repel the beetle.
- Various installations of MiteGone pads are successful in repelling the beetles in fall and winter for at least twelve weeks.
- None of the applications of MiteGone pads used in trial attracted the beetle into the hives.

While the above answers provided in this trial are of great importance, more secondary questions are arising and are a subject of the following discussion.

DISCUSSION AND RECCOMENDATION FOR PHASE II

TREATMENT, TIMING, AND WEATHER: While in this trial the treatment dates achieved were almost perfect. (see Tables 1 and 3). At the beginning of the trial on November 7, colonies had nine frames of brood. Pads evaporated in twenty-eight days and on December 12, the 35 day the end of the primary treatment period, the Colonies had only 2 to 4 frames of brood. Damage from mites was prevented from occurring in brood of winter bees by reducing the population of varroa by 80-85% and making surviving mites sick and incapable of reproduction before the winter brood vas laid.

Question Arising: *Is November 7 to December 12 the right treatment period?* YES for 2007, NO in general. Fall of 2007 was warm and ***late*** year. The temperature in the Gulf of Mexico was 70°F until December 12, while in normal or colder years the temperature of the gulf drops below 68°F by Nov. 20th Bees do not work and advance the production of brood by the human calendar but by Mother Nature. In cool years our trial would have been late., Treating earlier does not possess problems and will provide the same good result of protecting winter bees. I recommend treatments to start as soon as the first of fall's dry and pleasant weather arrives. According to my fifteen years of records this occurs around October 15.

Question Arising: *What would be the perfect timing and treatment for the spring?* From weather records we know that mid-April to May is the driest and nicest weather in this subtropical region. While in the northern climate we treat when apricots bloom and before mites can damage summer bees. That is before the increase into the full brooding period, when colonies have three to five frames of brood and are prepared for pollination. In a subtropical climate, this would happen in February and this is too soon for semiannual treatment. But in April and May colonies will have a full box of nine frames of brood and will be back from citrus pollination, strong and blooming, giving varroa plenty of opportunities to reproduce and hide in the capped brood. Sufficient dosage and length of treatment must be applied to keep varroa below the economic damage threshold, which we consider as being below eight to ten mites natural drop (Ruzicka Handbook of Testing and Test Results at www.mitegone.com).

Question Arising: *Will the treatment with two half-pads be sufficient to sustain mite levels below the economical damage threshold until fall treatment as it does in the northern climate and is recommended in present instructions?* Twelve years of experience shows that :
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EFFECTIVE, NON-WEATHER DEPENDANT MITEGONE FORMIC ACID TREATMENT:

Requires the delivery of 6 grams of acid per day for every 5 frames of bees in a standard deep Langstroth hive. One pad's evaporation surface will achieve this rate at 75°F / 24°C and 55% humidity (the same temperature and humidity that the bees maintain inside the hive at pad location between the frame and the wall in moderate climates). What happens if during your treatment, your hives cluster and stop maintaining the temperature and humidity in the hive? Or, what happens if the outside temperature is over 86° F/30° C, and outside humidity is 90%? The bees do not have a way of reducing humidity, so what happens when the humidity inside the hive reaches 90%? The answer to both of these questions is that nothing happens. The acid does not evaporate nor do mites get killed. Your treatment is simply on hold. **When conditions improve, acid will evaporate at a constant rate and mites will drop down into pool of heavy acid concentration and die.** In reality a half pad treatment can last a month or longer and a full pad treatment can last 60 or more days (Ruzicka et al. Handbook of Treatments, 2004).

Recommendation: In Phase II. We shall treat Group B with three half-pads and Group A with two half-pads to find which application or dosage increase is required for subtropical spring treatment.

HONEY CONTAMINATION, ORGANIC PRODUCTION, and IPM: Most countries consider treatment with formic acid the only acceptable treatment for organic production. "Formic acid, because it is already a natural constituent of honey, was exempted by the EPA from tolerance level studies. In its approval, the agency states: "Because there are essentially no residues resulting from the use [of formic acid], the EPA believes there are no dietary risks concerns with such use" (Simanuki, Fedlaufer, Apiculture Registration). European countries treat in mid-summer without any concern of contamination (Vesley, V. Formidol Czech Research Institute). One Florida beekeeper, using formic acid during Brazillian peper and Malaluca flow declared that that honey does not sting so badly anymore.

Because removing and extracting honey in the fall is no problem it is just a question of management. The spring situation may pose contamination question, especially if the top application where acid is poured and evaporated on the top of frames of the top box in high rates.

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The MiteGone method evaporates a miniscule amount of acid continuously at the bottom of pads, which when installed in the super above the queen excluded after extraction. The acid actually evaporates at the top of the brood chamber and because it is heavier than air it flows down toward the bottom boards and possesses little opportunity to mix with honey in supers. Regardless of all the above, I recommend that a test of honey contamination should be part of Phase II during spring treatment.

I PM

Until bio pesticide controls such as funguses (Ruzicka, James, Kanga et al, 1992-2008) are available as a possible semi annual treatment companion for formic acid to control varroa and trachea mite, the only other practical treatment for the commercial operator is to use screen bottoms with removable "food" trays (trays which will collect concentrated fumes of acid.) These may also work in beetle deterrent repellent applications, open or leaky screen bottoms defeats the principle of evaporation treatments.

VARROA CONTROL

Only natural drop before the treatment in April will confirm the success of the treatment in the fall.

Group A & B: The evaporation surface of the MiteGone pad, regardless of its length, is 3/8 " thick and 4" wide. Under the standard temperature of 75^0 F and 55% humidity it will evaporate 6 grams of acid in low continuous flow over 24 hours, The purpose of these groups were to verify if we reached the target of 6 grams per pad within a period of a minimum of 21 days.

Group B 60 grams evaporated from each quarter pad in less than14 days, at 10 days would be 6 grams, in 14 days 4.8 grams.

Group A: 120 grams evaporated from each half pad in less than 28 days. In 21 days 5.7 grams had evaporated and at 28 days 4.3 grams. Statistically, both groups reached the evaporation target.

INCREASE OF DOSAGE Group B:

Group B, by using four evaporating surfaces delivered 30 % more acid per day as did group A using 3 evaporating surfaces. Table I Results:

The efficacy multiple of Group A: Bill 7.8, Steve 8.73 is higher than the efficacy multiple of Group B: Bill 6.9, Steve 7.95, proving that the increase of acid delivery per day will not improve efficacy, and 3 half pads of STD MiteGone treatment will provide sufficient strength and length of treatment.

Questions to be answered in Phase 2

*Will the efficacy multiple of 2 pads match the 3 pad instillation in April?

*What will be the level of mites in all groups by verification natural drop tests before treatment in April?

*Group C With an advantage of having 30% less mite than Groups A,B, and D, what level will the mite population in Group C grow to, and will it surpass the economic damage threshold of 10 mites in a 24 hour prorated natural drop?

SMALL HIVE BEETLE

It is important to remember that the repellent ability is unique to MiteGone methods and pads only unlike other applications of Formic Acid which are being blamed for attracting SHB into hives (Westerweld, Mendez, et al)

We demonstrated that no MiteGone treatment attracts the beetle into the hives; on the contrary **group E** successfully repels it for 12 weeks.

We also demonstrated that standard treatment will repel the beetle for 7 weeks; However, it was only on 2 hives this fall and does require further tests. The proper test on infested hives is required in Phase 2 and next fall in Phase 3.

Another question is how all repellent applications will perform in the rainy part of the year during the June to October period and what would be the success of the vertical pad installation?

REPELLENT METHODS: Shown on page 7 . (Ruzicka, *Manual of Treatments 2004)*

Repelling ability or improvement of screen bottoms efficacy by MiteGone was known to the writer since 2004 through exploratory tests in Florida and other parts of North America. But lack of suitable cooperators led to exploratory small scale trials only.

In this trial we eliminated the possibility of pads installed on skewers acting as a beetle trap. No beetles in Group D or E were attracted to hide under the pads and be affected by acid vapors. The same installation on Group E repelled the beetle and kept it out for 12 weeks. The final length of effect will be known in Phase II.

The installation of the pad over the skewers on the bottom board was designed to increase the rate of evaporation and prevent the pad from being soaked with rain water. Laying the pad on the bottom was originally used in trays of screen bottom boards to improve their efficacy.

Placing the pads on the bottom board often results in propolising which may or may not affect the repelling abilities of the installation. The bottom installation may be best to use in conjunction with screen bottoms with food trays (such as are used in hospitals) where the pads are protected from propolising by bees.

The vertical pad installation with a cut corner was the most promising in previous exploratory tests, but was not included in this trial. It needs to be tested in all applicable weather conditions in Phase II. and possibly Phase III..

AVAILABILITY

The last major obstacle is the availability and practicality of preparing pads and doing actual treatments by various levels within the beekeeping community while large commercial operation and sideliners with more than 50 hives will opt to buy acid and dry pads commonly available in most states, and train themselves in the safe use of it in exchange for a low cost . A commercial operator can keep his hives clean of most mites and beetles for $3 a year.

However, the above approach is not very suitable for part timers and hobby beekeepers. Pre filled products exist. Manufacturing methods and shelf life were tested, sold and used in Canada since 2004. All the yearly tests done by our research has been done using these pre filled pads. The last batch was made in 2006 and is shown in the picture. However, use and availability in the USA requires a US entity to register this pre filled product.

Make it and sell it. MiteGone now sells these kits in dry form only. Customers need only add 2.4 Kg of 65% Formic Acid or 2.5 Littre by volume. Gloves and respirators are recommended for application and filling.

In Conclusion, MiteGone Formic Acid delivery system has been proven to provide a suitable solution to varroa and beetle control in a fall application in subtropical climates. Hopefully, we can provide in Phase II the same results in the spring and summer applications. This will keep down the levels of both pests and will provide a viable solution for hobby and commercial beekeepers of the Southern United States as it has already provided for the Northern Temperate climate.

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