



Arista Bee Research

Foundation for breeding varroa resistant honey bees



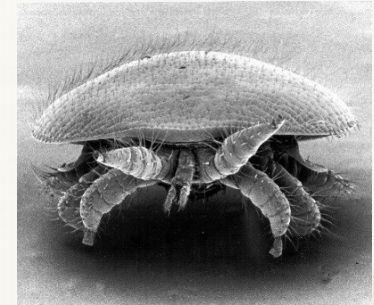
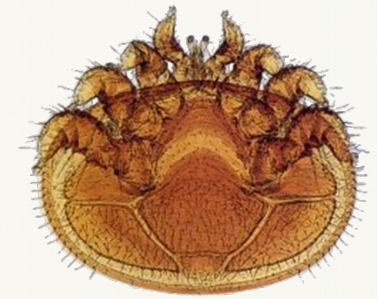
L'élevage d'abeilles VSH dans les traces de John Harbo

Renaud Lavend'Homme &
Bartjan Fernhout
Clermont-Ferrand, Octobre 2016
Version 1fr



Varroa

Varroa destructor



- Le Varroa affaiblit l'abeille en se nourrissant de son hémolymphe et cause de sérieux dégâts à l'abeille en propageant des virus et des bactéries.
- Les colonies non traitées s'effondrent dans les 2 ans des conséquences de la croissance rapide de la population de Varroas.
- Le traitement des colonies est laborieux, a des résultats variables et n'affecte pas seulement les acariens, mais nuit également aux abeilles.
- Le Varroa est considéré comme étant le principal responsable de la mortalité (hiver).

Varroa

Varroa destructor



Varroa

Varroa destructor



- Les populations d'abeilles, importantes pour la pollinisation de nos cultures, sont mises sous pression par une maladie grève causée par un acarien : Varroa Destructor. L'abeille européenne n'est pas (encore) capable de vivre avec cet acarien.



Varroa destructor is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada*

Ernesto GUZMÁN-NOVOA¹, Leslie ECCLES¹, Yireli CALVETE², Janine MCGOWAN¹, Paul G. KELLY¹, Adriana CORREA-BENÍTEZ²

Table II. Mean fall conditions (\pm SE) of 408 honey bee colonies found alive or dead the following spring in Ontario, Canada, for different factors that could be associated to colony mortality and low spring and summer bee populations. *P* based on Mann-Whitney U tests.

Factor	Alive	Dead	<i>P</i>
Bee population ¹	8.0 \pm 0.1	5.9 \pm 0.2	< 0.0001
Food reserves (kg)	25.4 \pm 0.5	19.0 \pm 0.9	< 0.0001
Varroa mite infestation ²	2.9 \pm 0.2	11.1 \pm 1.5	< 0.0001
Tracheal mite infestation ³	0.2 \pm 0.1	3.1 \pm 1.5	< 0.001
<i>Nosema</i> infection ⁴	6 734 \pm 3 238	18 018 \pm 14 923	0.2847

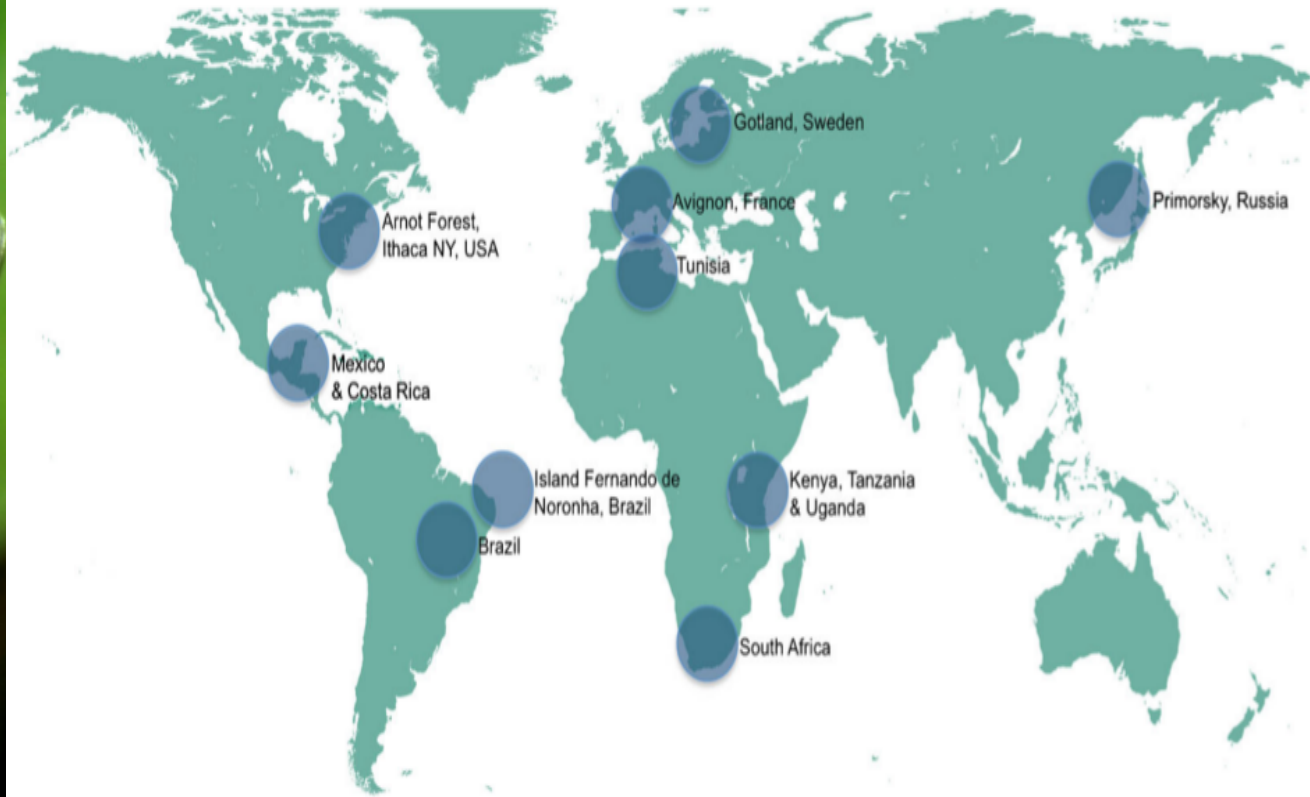
¹ Number of frames covered by bees per hive; ² number of mites per 100 bees; ³ number of parasitized bees in 100; ⁴ number of spores per bee.

Varroa Sensitive Hygiene

*Un comportement naturel
de résistance à Varroa*

... **que faire???**

comment la nature **Nature** résout ce problème?
=> développement de **Résistance à Varroa**



Varroa Sensitive Hygiene

Natural behavior to withstand Varroa

Honeybee *Apis Mellifera*

Development from egg to adult, with Varroa Mite

Queen laying egg.....

Worker Bee feeding Larva.....

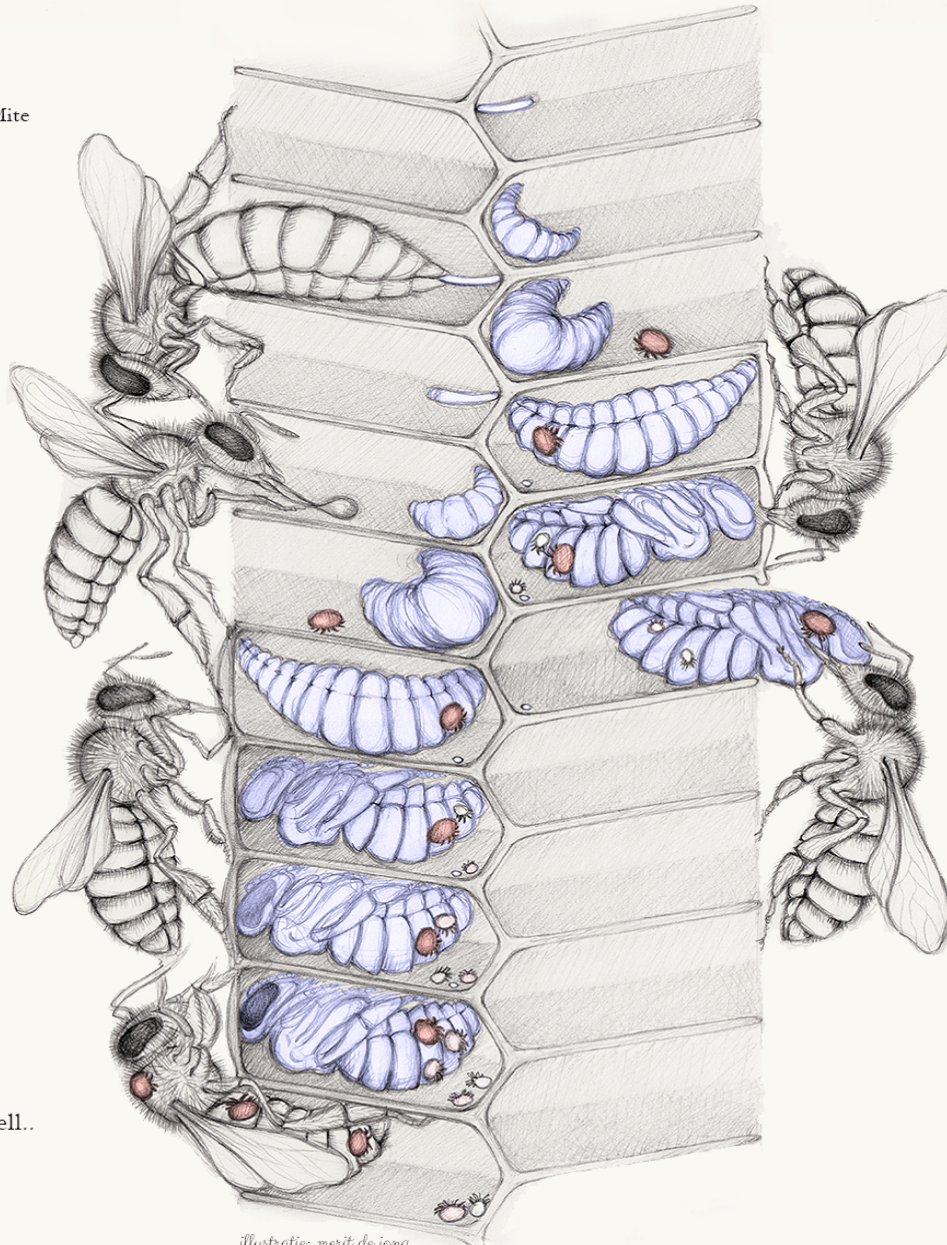
Varroa enters cell with Larva.....

Larva reaches full growth.....
Worker Bee closing cell.....

Reproducing Varroa mite.....

Pupa developing.....

Young Bee with Varroa mites leaving cell..



Varroa resistant honeybee

Varroa Sensitive Hygiene Behavior

.....Larva






.....Varroa enters cell with Larva

.....Reproducing Varroa mite

.....Worker Bee making hole in cap

Worker Bee removing Pupa and mites

Varroa Legenda:

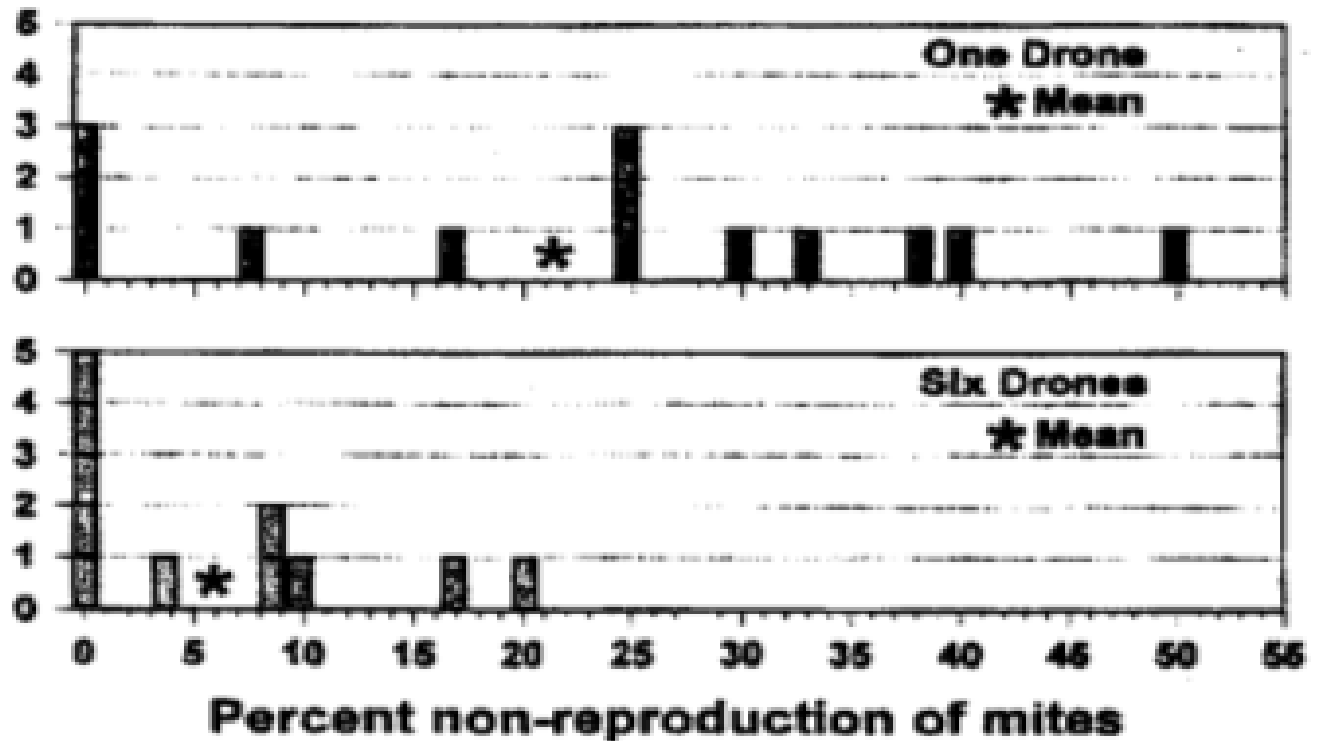
- Adult female
- Adult male
- Egg
- Protonymph
- Deutonymph



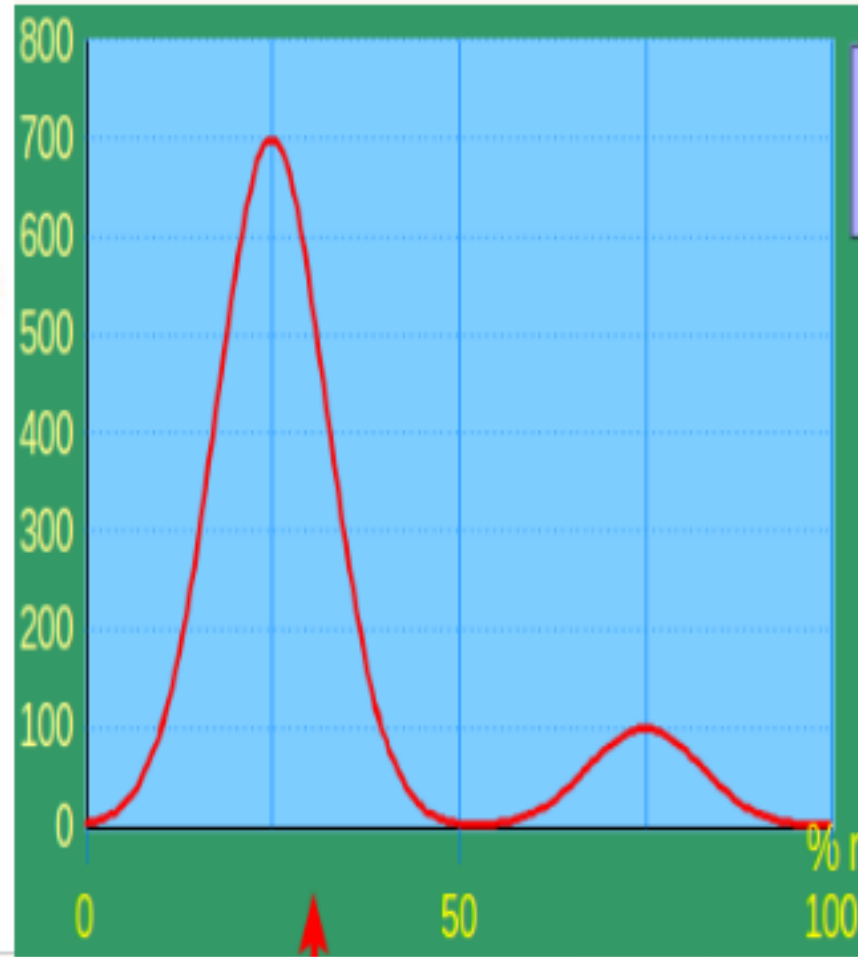
The value of single-drone inseminations in selective breeding of honey bees

JOHN R. HARBO

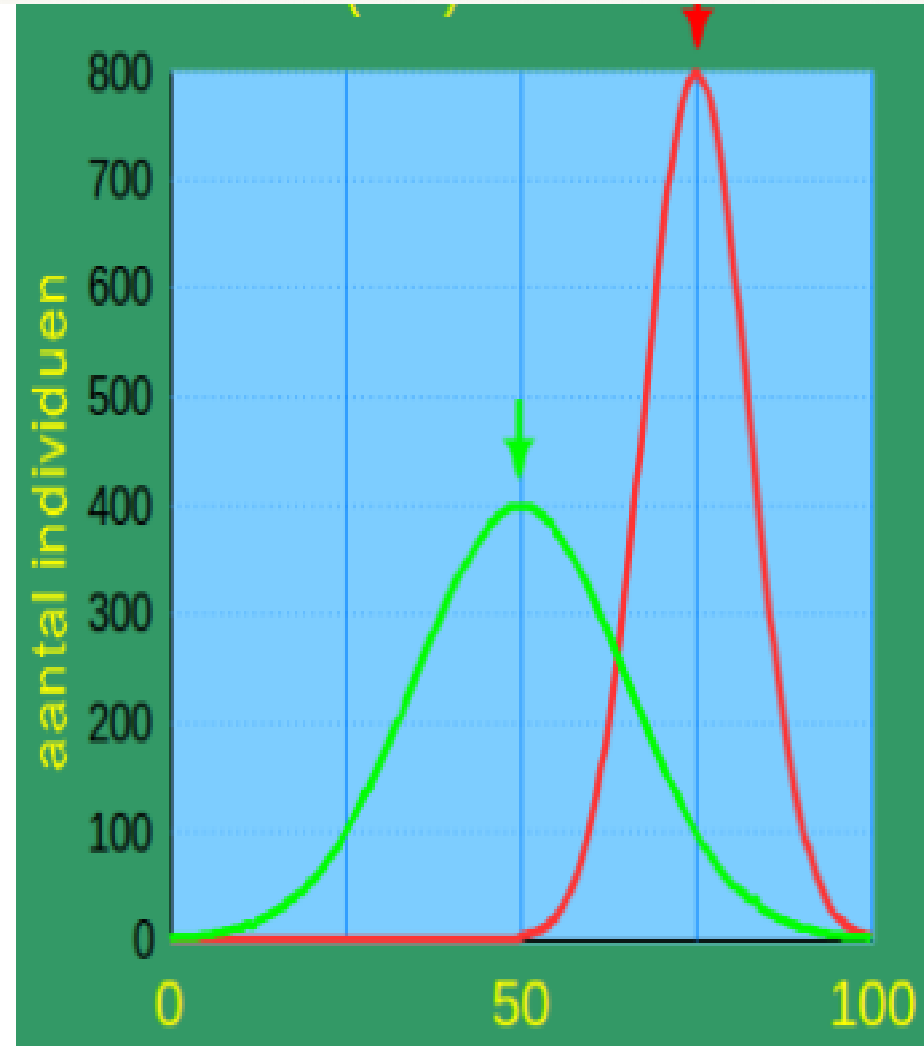
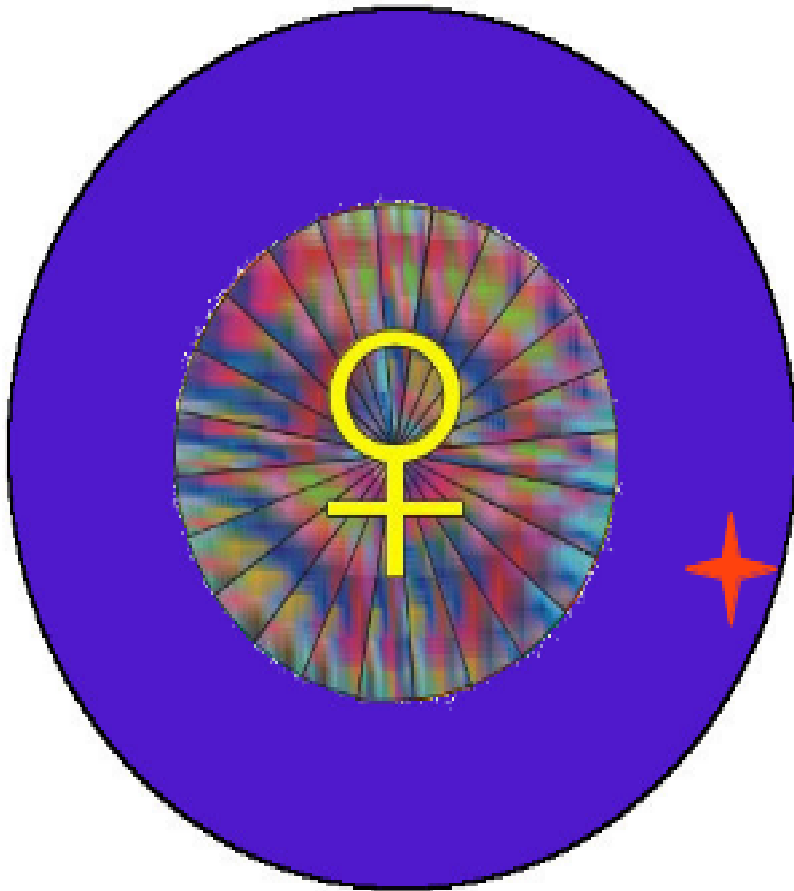
HONEY BEE BREEDING, GENETICS, & PHYSIOLOGY LABORATORY
AGRICULTURAL RESEARCH SERVICE, USDA
1157 BEN HUR ROAD, BATON ROUGE, LA 70820



proximité génétique : fécondation multiple



proximité génétique : fécondation par 1 mâle



Honey Bees (Hymenoptera: Apidae) in the United States That Express Resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae)

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Honey Bee Breeding, Genetics and Physiology Laboratory, USDA-ARS, Baton Rouge, LA 70820

J. Econ. Entomol. 90(4): 893-898 (1997)

ABSTRACT The purposes of this study were to select honey bees, *Apis mellifera* L., for resistance to varroa mites, *Varroa jacobsoni* Oudemans, and to find a probable cause for this resistance. As a genetic source, we assembled 8 colonies that we thought had potential for resistance to varroa. Queens and drones were propagated from this group to produce 43 instrumentally inseminated queens, each queen mated to only 1 drone. Colonies from 27 of these queens were tested in Louisiana and 16 were tested in Michigan. Each colony in the Louisiana test began with 986 ± 13 g (mean \pm SD) of bees and ≈ 290 mites; Michigan colonies began with $3,212 \pm 171$ bees and ≈ 51 mites. The populations of mites and bees were measured 10 wk later. Three of the 43 colonies had fewer mites at the end of the test than at the beginning. During the experiment, we evaluated each colony for grooming behavior, hygienic behavior, the duration of the postcapping period, and the frequency of nonreproducing mites in brood cells. Of these 4 characteristics, only nonreproduction of mites was highly related to a change in the mite population. The duration of the postcapping period was marginally related, and the other 2 characteristics were apparently unrelated to the growth of the mite population. This study showed that resistance to varroa mites is present in the honey bee population in the United States, nonreproduction of mites was highly correlated with the growth of a mite population, and nonreproduction of mites may be a valuable characteristic for selecting bees for resistance to varroa mites.

KEY WORDS *Apis mellifera*, *Varroa jacobsoni*, breeding, selection

ORIGINAL RESEARCH ARTICLE



Bees with *Varroa* Sensitive Hygiene preferentially remove mite infested pupae aged \leq five days post capping

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Summary

Suppressed Mite Reproduction (SMR) is a trait of honey bees that provides resistance to *Varroa destructor*. The mechanism of resistance in SMR bees is the removal of infested pupae from capped brood, so a better name is VSH bees (acronym for *Varroa* Sensitive Hygiene). This study compared the removal of infested brood by VSH and control bees to determine whether VSH bees removed infested pupae of different ages at similar rates. A pair of infested combs containing all stages of pupae were transferred into each host colony (six VSH and six control colonies) for 40 hours. VSH bees removed significantly more (55%) infested cells (singly and multiply infested), than controls (13%). They removed significantly more (66%) singly infested pupae aged from one to five days post capping (cohort A) than did controls (16%). The two types did not differ in the removal of singly infested pupae aged five to 10 days post capping (cohort B) (5–22%). Many pupae were found in uncapped cells at the end of the test, and most of the uncapped pupae were infested with mites. None of the uncapped cells contained prepupae, the development stage occurring during the first three days post capping. Thus, removal of infested pupae may be triggered by stimuli in cells with pupae aged 3–5 days post capping.



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Lignée VSH



Pol-line



Non-résistantes



2 populations

**Abeilles
non-résistantes**



**95% mortalité
sans traitement**

**Abeilles
POL-line**



**15% mortalité
sans traitement**



*Breeding, Selection &
Distribution*



Expertise VSH : USDA Baton Rouge



Bob Danka



Philip Tokarz



John Harbo



Garrett Dodds



Projects

Breeding, Selection & Distribution

Groupes de Travail



Buckfast BE-LU-FR



Carnica Lux



Altea Breeding SP



Carnica NL



Italian US-Hawaii



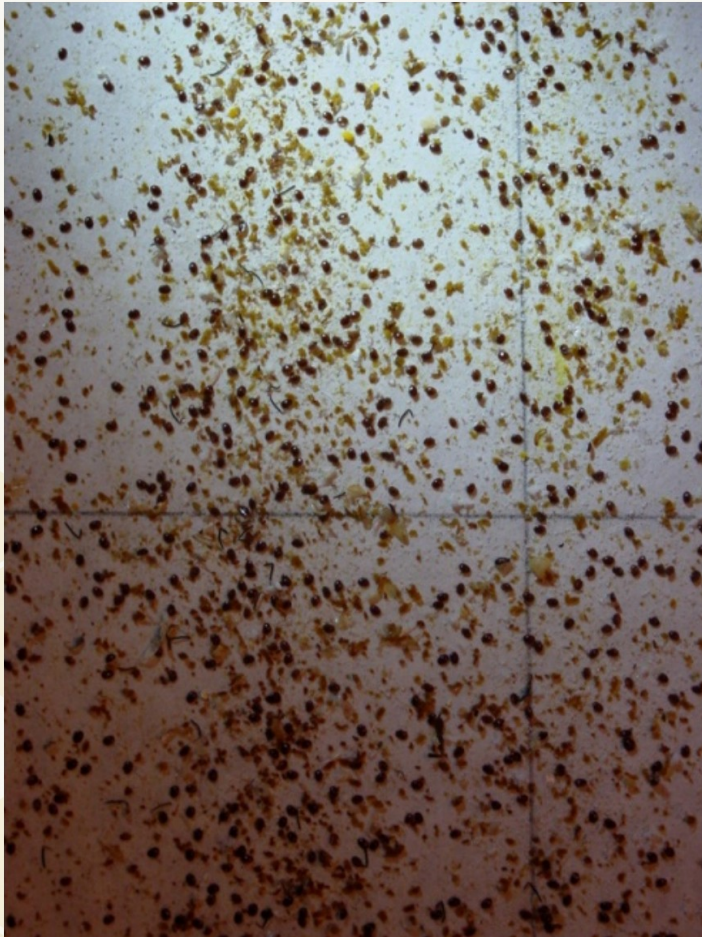
Buckfast Bavaria DE



Buckfast NL

*Breeding, Selection &
Distribution*

Méthode: 1. Pré-sélection des colonies



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Méthode : 2a. Création de colonies-test



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Méthode : 2b. Insémination à 1 mâle (SDI)





Breeding, Selection & Distribution



Méthode : 3. Infestation avec des Varroas supplémentaires







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Méthode : 4. Comptage VSH







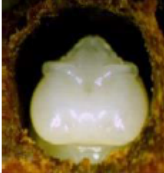

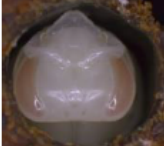




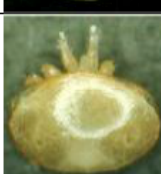


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Méthode : 4. comptage VSH

Stages of honey bee brood and mite offspring for normally reproducing Varroa

Days post capping	Brood stage		Eldest mite offspring	
3		prepupa		Varroa egg
4		pupa, white eyes		first proto-nymph (male)
5		pupa, pink eyes		second proto-nymph (female)
7		pupa, purple eyes		first female deutonymph
10		pupa, brown head, black eyes		first adult daughter

*Breeding, Selection &
Distribution*

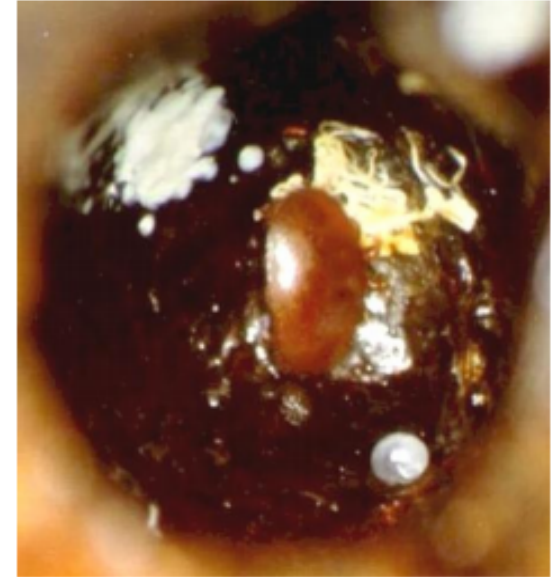


Méthode : 4. Comptage VSH

Optimal brood stage: 7 days post capping or later



**adult female with offspring
(= normally reproductive)**




**adult female mite with progeny
that are too late to mature
(= non reproductive)**

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Method: 4. comptage VSH

VSH Scoring form - Reproduction of Varroa								
Test colony:			Operator:			Date:		
Pedigree:								
Brood stage:		0 larva/prep.	1 white eyes	2 pink eyes	3 purple eyes	4 black eyes		
Infested cell	Investigated cell no	No of foundress mites	Adult daughters (no)	Deutonymph (no)	Protonymph female (no)	Male (no)	Brood stage (see above)	Single infested, non reproductive
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								

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Method: 4. comptage VSH

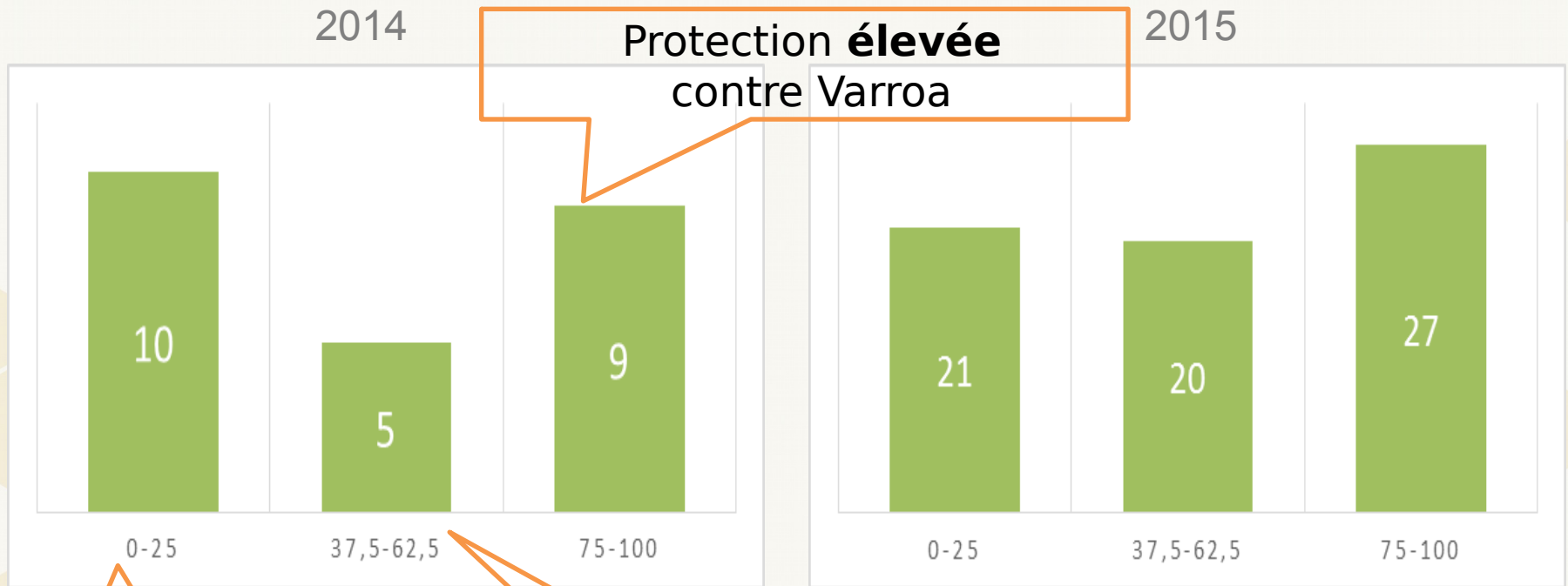
	Control	Colony 1	Colony 2	Colony 3	Colony 4
Mite 1	NR	NR	NR	NR	NR
Mite 2	NR	NR	NR	NR	NR
Mite 3	R	R	R	R	
Mite 4	R	R	R	R	
Mite 5	R	R	R		
Mite 6	R	R	R		
Mite 7	R	R			
Mite 8	R	R			
Mite 9	R				
Mite 10	R				
Non-Reproducing mites	2	2	2	2	2
Reproducing mites	8	6	4	2	0
NR%	20%	25%	33%	50%	100%
Removed reproducing mites	0	2	4	6	8
%Reproducing removed, %VSH	0%	25%	50%	75%	100%

Breeding, Selection & Distribution



Résultats SDI: background USDA

Matériel génétique créé à partir de buckfast inséminées 2x consécutivement avec du sperme VSH (75% USDA-VSH)



% VSH, # Colonies.

Peu ou pas de protection contre Varroa

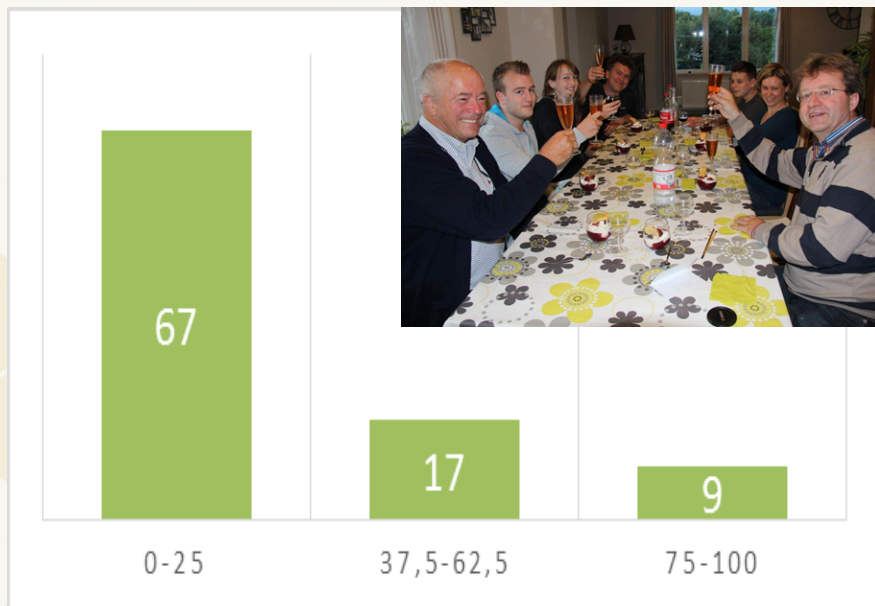
Protection **Modérée** contret Varroa

Breeding, Selection & Distribution

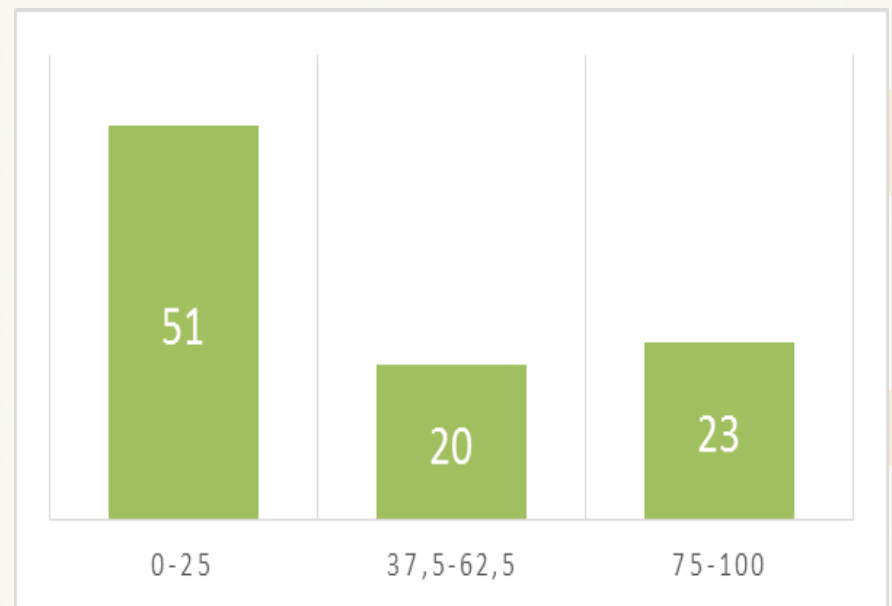


Résultats SDI: Buckfast

2014 (1st année)



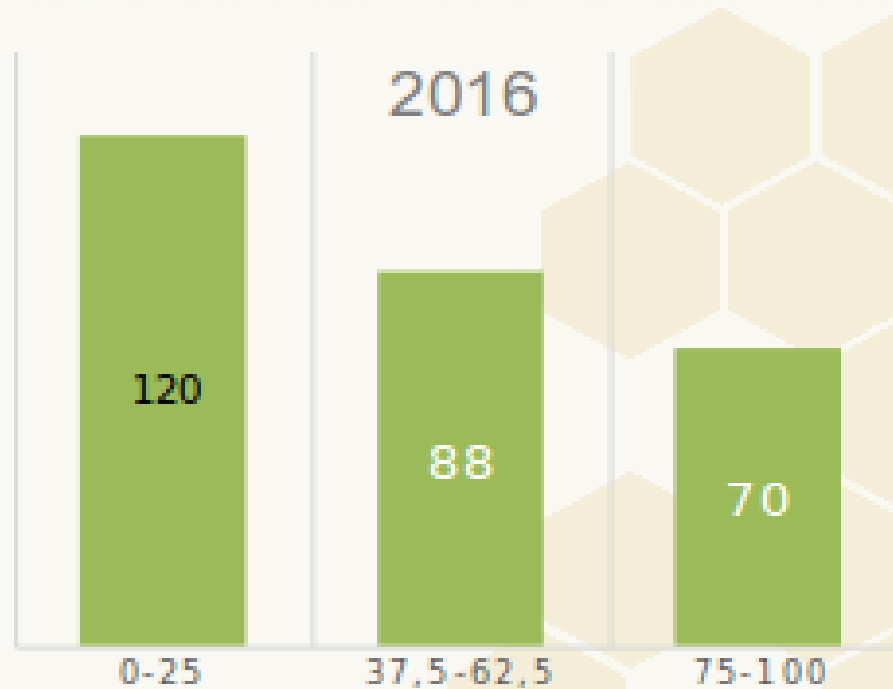
2015 (2nd année)



*Breeding, Selection &
Distribution*



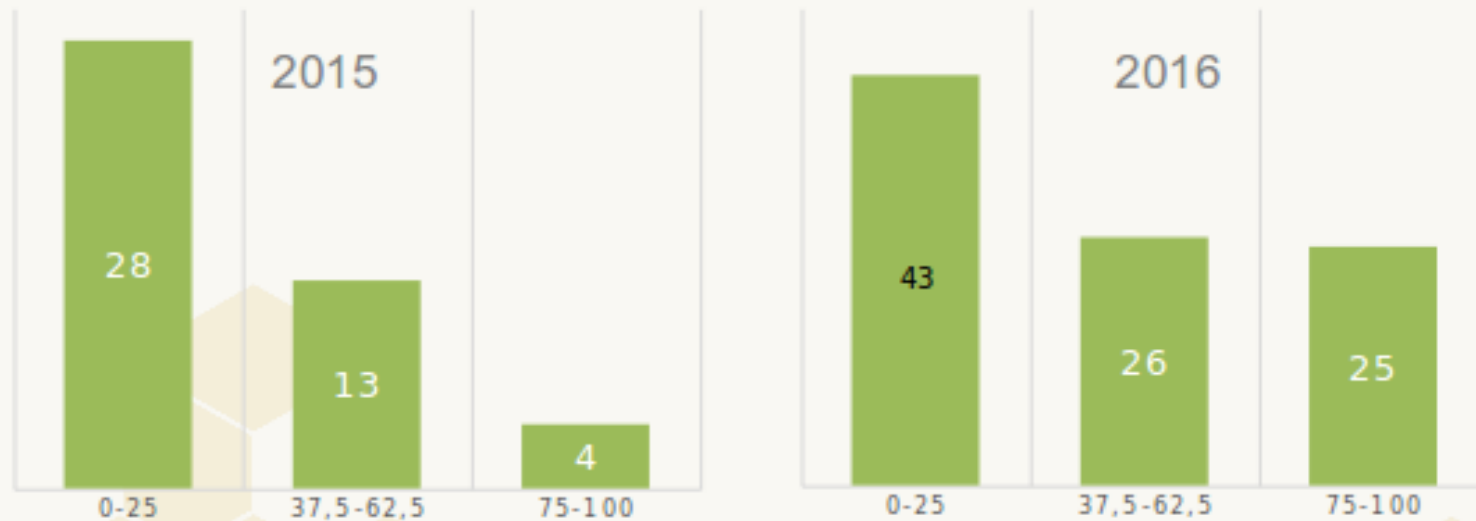
Résultats SDI: Buckfast



Projects

Breeding, Selection & Distribution

Results SDI: Carnica



Sample size, avg # of cells (mites) investigated.

0-25 37,5-62,5 75-100

2015 165 (14) 275 (15) 293 (15)

2016 183 (121) 234 (15) 298 (13)



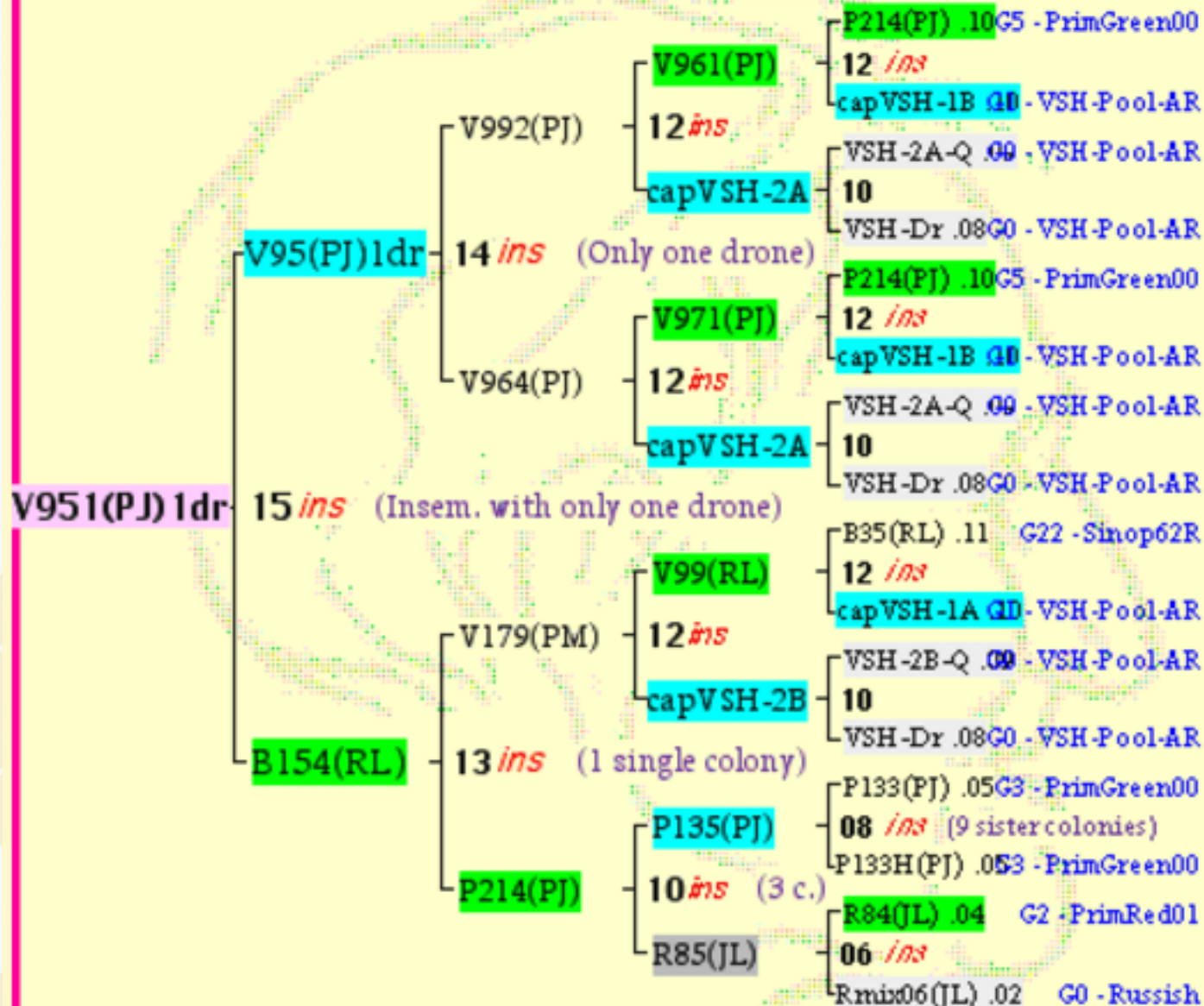
Apis mellifera carnica

Breeding, Selection & Distribution



Method: 4. comptage VSH

	Control	Colony 1	Colony 2	Colony 3	Colony 4
Mite 1	NR	NR	NR	NR	NR
Mite 2	NR	NR	NR	NR	NR
Mite 3	R	R	R	R	
Mite 4	R	R	R	R	
Mite 5	R	R	R		
Mite 6	R	R	R		
Mite 7	R	R			
Mite 8	R	R			
Mite 9	R				
Mite 10	R				
Non-Reproducing mites	2	2	2	2	2
Reproducing mites	8	6	4	2	0
NR%	20%	25%	33%	50%	100%
Removed reproducing mites	0	2	4	6	8
%Reproducing removed, %VSH	0%	25%	50%	75%	100%





Bees
à l'air
de l'air



216

215



- | Comparez ici :
- ▣ Le même jour une ruche traitée normalement!

V179(PM) x P214(PJ) : 250v

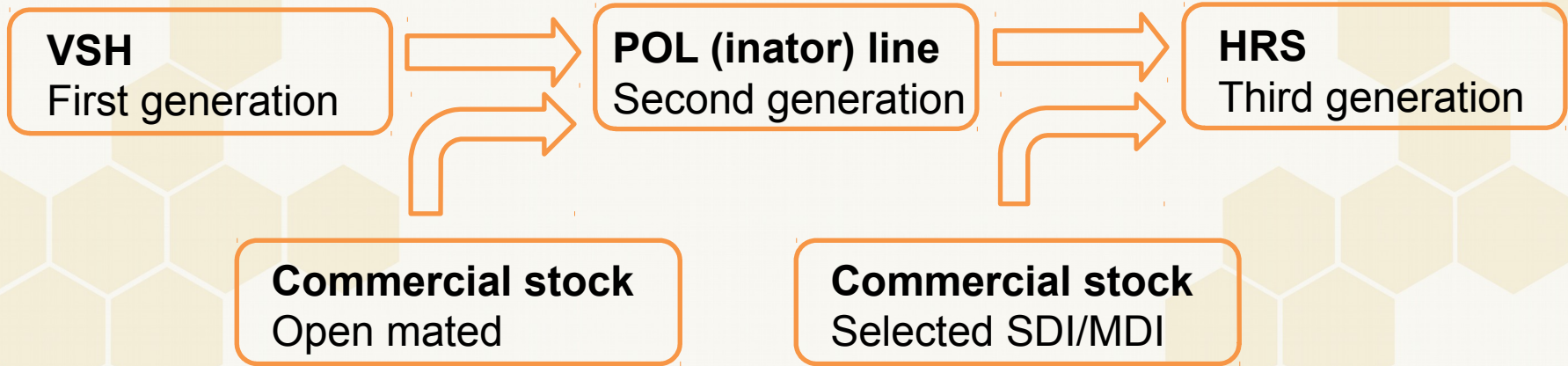
Buck x Buck : >2000v



(n) mites after 48 h:	10.5	39.3	67.6	155.1	78.9	61.2
(n) mites after 14d:	2.4	13.5	2.9	16.2	10.7	9.2
(n) mites total:	12.9	52.8	70.5	171.2	89.9	70.5
SD:	16.1	51.4	77.5	239.7	63.9	117.9
Maximum (n) of mites:	53	157	269	798	235	

US Varroa resistant honey bee

Breeding, Selection & Distribution
Hawaii Resistant Stock



Breeding, Selection & Distribution



Conclusion et perspective

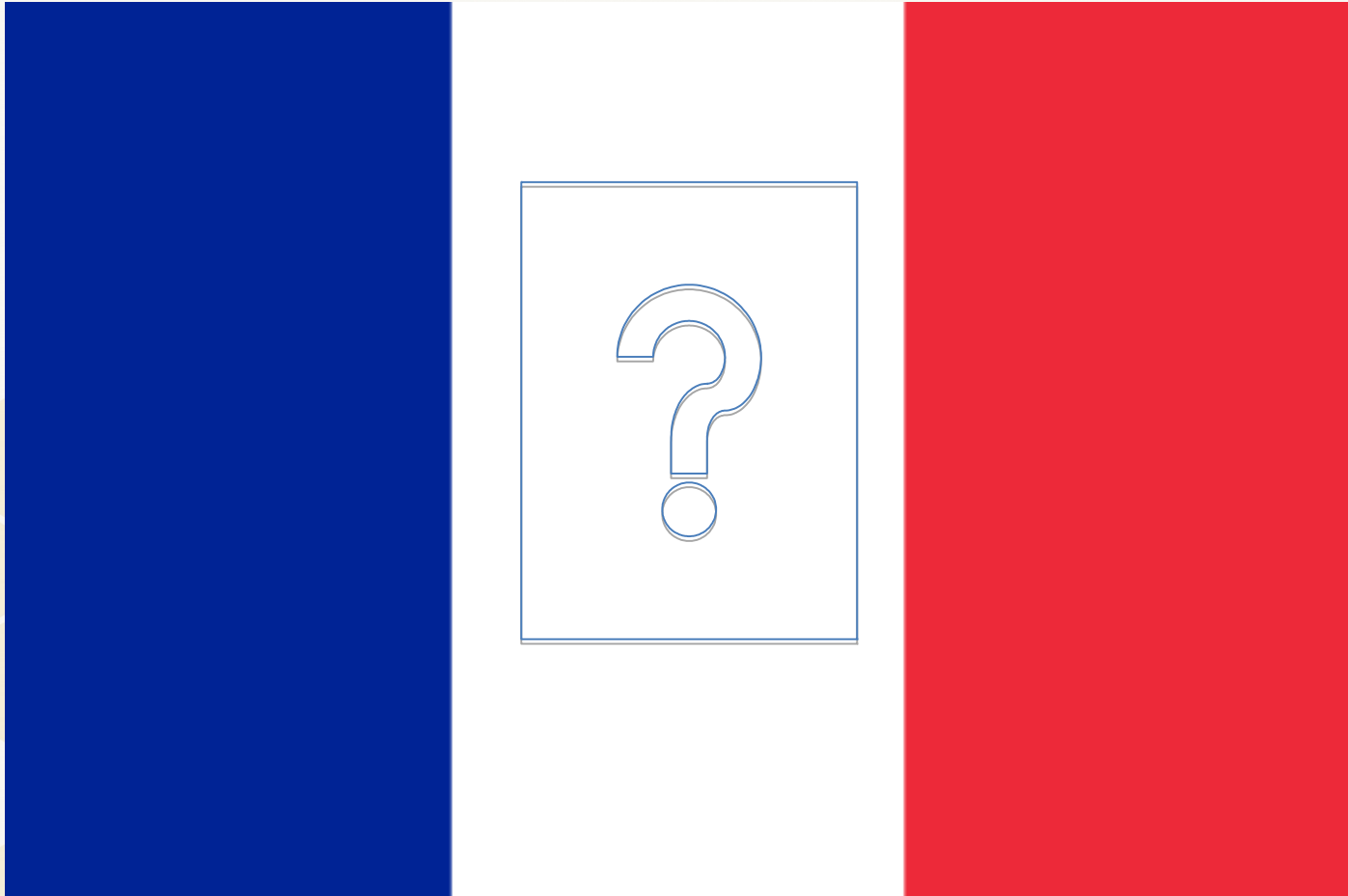
- La Fondation Arista Bee Research est active en BE, NL, Lux, DE et USA.
- La Fondation Arista Bee Research a repris (et amélioré) les techniques développées par John Harbo et les a implantées en Eu.
- Le comportement VSH, originellement mis en évidence dans des lignées de l'USDA fonctionne également dans nos mains en Europe.
- Le comportement VSH est également présent dans nos lignées d'abeilles européennes et il peut être porté à un niveau élevé grâce à la technique d'insémination à 1-mâle.
- La Fondation Arista Bee Research peut annuellement générer plusieurs dizaines de colonies SDI hautement VSH.

*Breeding, Selection &
Distribution*
Conclusion et perspective



- Ce travail doit être répéter sur autant de lignées d'abeilles que possible (races et lignées) pour assurer une base génétique forte et une biodiversité qui rendra possible une utilisation large dans la communauté des apiculteurs.
- Le travail d'élevage dans le futur doit se focaliser sur l'obtention de colonies VSH 100 % dans des lignées sans liens de parenté.
- Obtention de colonies 'normales' (=inséminées avec plusieurs mâles) de production ainsi que l'évaluation des autres comportements non VSH (miel, fécondité, douceur, tendance à l'essaimage etc).
- Une haute priorité est donnée au développement des marqueurs génétiques pour le VSH. Ceci est important pour une acceptation large et une implémentation dans les programmes d'élevage.

Breeding, Selection & Distribution

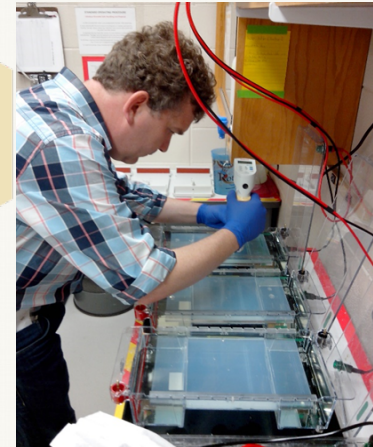


Breeding, Selection & Distribution

Les marqueurs génétiques



Bob Danka



Bas Zwaan
Bart Pannebakker



Frens Pries



Le Budget

*Ce que nous avons
besoin....*



Niveau financier actuel:

- 11500 euros/an de donateurs européens.
- 36000 dollars/an de donateurs US.
- 50000 dollars de l'USDA.

Nous souhaiterions atteindre 1.2 mln euros / an (5 salariés, 45 volontaires, 5 étudiants, 1 PhD).

Votre soutien sera apprécié !

A propos de nous

Qui participe....

Les volontaires

Riad Abara, Herman Arts, José Artus, Bart Barten, Erol Bilen, André Bosseaux, Pascal Boyard, Didier Bricks, Christane Büchler, Gust Cordewener, Jean-Paul Demonceau, Jean-Pol clause, Jacques Delhez, Jean-Marie Desaulty, Michael Desaulty, Achiel Dhooge, Jean-Marie van Dijck, Achiel Dhooge, Vincent Driest, Nico Florissen, Didier Geuten, Rene Genet, Céline Gobin, Herman Groen, Jos Guth, Selma Hensen, Ralf Höling, Christan Hupfer, Mari van Iersel, Paul Jungels, Victor Jungels, Margaux Jungels, Nico Kalmes, Jan Keemink, Martin Klein, Thomas Kodym, Josef Koller, Pierre de Koning, Gerbert Kos, Jan Lagerweij, Philippe Lambert, Michel Leloup, Jean-Marie Lavend'Homme, Renaud Lavend'Homme, Bernard Leclercq, Marwim van Limburg, Sefan Luff, Pierre Marin, Charles-Louis Maudoux, Hans Middelbeek, René van der Molen, Joé Molitor, G. Morris, Isabelle Matis, Charles-Louis Moudoux, Peter Oudshoorn, Julien Perrin, Ireen Roskam, Bernard & Françoise Sauvager, Jeanne van Sebille, Daniel Schuster, David Thomas, Guus Verhoeven, Tieme Wanders, Fritz Zieher.

Le comité scientifique et les conseillers

Kaspar Bienefeld, Ralph Büchler, Prim Brascamp, Bob Danka, Danielle Downsey, Tom Rinderer, David Thomas, Bas Zwaan, Piet Boonekamp, Marcel Dicke, John Harbo, Jeffrey Harris, Ina Heidinger, Bart Bannebakker, Frens Pries.



Le conseil d'administration

Danny Goovaerts, Marcus Gravendyck, Rolf Wildeman, BartJan Fernhout

Questions ??

To bee or not to bee...VSH !



<http://aristabeereseearch.org>

Cimetière de slides

VSH215= .13-VSH992ins A199 (Insemination normale)







Queen	Drone	Workers
4	2	100
4	1	75%
4	0	50%
3	2	100%, 75%
3	1	75%, 50%
3	0	50%, 25%
2	2	100%, 75%, 50%.
2	1	75%, 50%, 25%
2	0	50%, 25%, 0%.
1	2	75%, 50%
1	1	50%, 25%,
1	0	25%, 0%
0	2	50%
0	1	25%
0	0	0%

OBSERVATIONS DES POPULATIONS D'ABEILLES ET DE *VARROA* DANS LES COLONIES À DIFFÉRENTS NIVEAUX D'INFESTATION

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F 31016 Toulouse Cedex

RÉSUMÉ

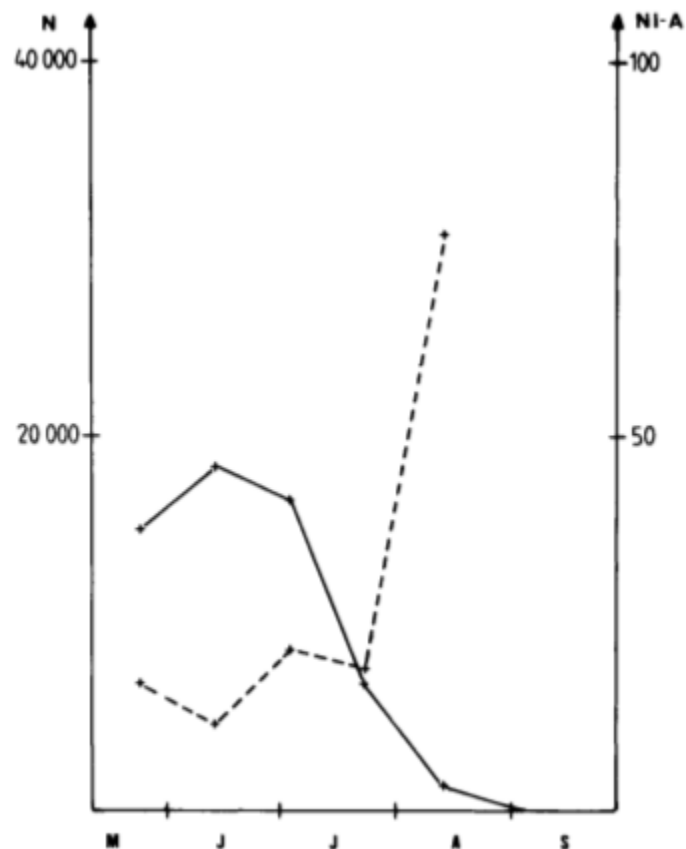


FIG. 2. — Population d'abeilles et leur niveau d'infestation dans des ruches fortement infestées

En ordonnées :

— N = nombre d'abeilles

- - - NI-A = niveau d'infestation des abeilles (%)

Global Honey Bee Viral Landscape Altered by a Parasitic Mite

Stephen J. Martin,^{1*} Andrea C. Highfield,² Laura Brettell,¹ Ethel M. Villalobos,³ Giles E. Budge,⁴ Michelle Powell,⁴ Scott Nikaïdo,³ Declan C. Schroeder^{2*}

Emerging diseases are among the greatest threats to honey bees. Unfortunately, where and when an emerging disease will appear are almost impossible to predict. The arrival of the parasitic *Varroa* mite into the Hawaiian honey bee population allowed us to investigate changes in the prevalence, load, and strain diversity of honey bee viruses. The mite increased the prevalence of a single viral species, deformed wing virus (DWV), from ~10 to 100% within honey bee populations, which was accompanied by a millionfold increase in viral titer and a massive reduction in DWV diversity, leading to the predominance of a single DWV strain. Therefore, the global spread of *Varroa* has selected DWV variants that have emerged to allow it to become one of the most widely distributed and contagious insect viruses on the planet.

The emergence of infectious diseases is driven largely by socioeconomic, environmental, and ecological factors (1), and these diseases have significant effects on biodiversity, agricultural biosecurity, global economies, and human health (2, 3). The honey bee is one of the most economically important insects, providing crop pollination services and valuable hive products (4). During the past 50 years, the global spread of the ectoparasitic mite *Varroa destructor* has resulted in the death of millions of honey bee (*Apis mellifera*) colonies (5). There is general consensus that the mites' association with a range of honey bee RNA viruses is a contributing factor in the global collapse of honey bee colonies (5–10), because the spread of mites has facilitated the spread of viruses (11, 12) by acting as a viral reservoir and incubator (13). In addition, the mites' feeding behavior allows virus to be transmitted directly into the bees' hemolymph, thus bypassing conventional, established oral and sexual routes of transmission. In particular, deformed wing virus (DWV) has been associated with the

collapse of *Varroa*-infested honey bee colonies (5, 8, 14–16), because it is ubiquitous in areas where *Varroa* is well established (6, 9, 17, 18). The rapid global spread of *Varroa* means that very little is known about the natural prevalence, viral load, and strain diversity of honey bee viruses before the *Varroa* invasion (15). Such data are important, because most honey bee viral infections were considered harmless before the spread of *Varroa* (9). Large-scale loss of honey bee colonies has been associated with viruses vectored by *Varroa* (5). The recent arrival and

subsequent spread of *Varroa* across parts of the Hawaiian archipelago has provided an opportunity to study the initial phase of the evolution of the honey bee–*Varroa*–DWV association. So far, colony collapse disorder (CCD) (6) has not been reported in Hawaii (19), but all of the associated pests and pathogens are present.

European honey bees (*Apis mellifera* L.) were first introduced to Hawaii from California in 1857. They were largely managed, but feral populations were soon established on every major island in the archipelago (20). Hawaii remained *Varroa*-free until August 2007, when the mite was discovered throughout Oahu Island. A subsequent survey by S. Nikaïdo and E. Villalobos during 2007–2008 recorded the collapse of 274 of 419 untreated colonies belonging to beekeepers. The disappearance of feral colonies from urban areas on Oahu was also noticed by beekeepers and pest control officers. Despite quarantine measures, the mite spread to Hilo on the Big Island in January 2009, where it survived an eradication attempt and by November 2009 had spread throughout the southern region of the island (Fig. 1). By November 2010, *Varroa* occurred throughout the Big Island. However, the islands of Kauai and Maui remained mite-free, and no unusual colony losses or disease problems have been reported there (19). The aim of this study was to investigate the influence that *Varroa* has in the spread of honey bee viruses during the initial

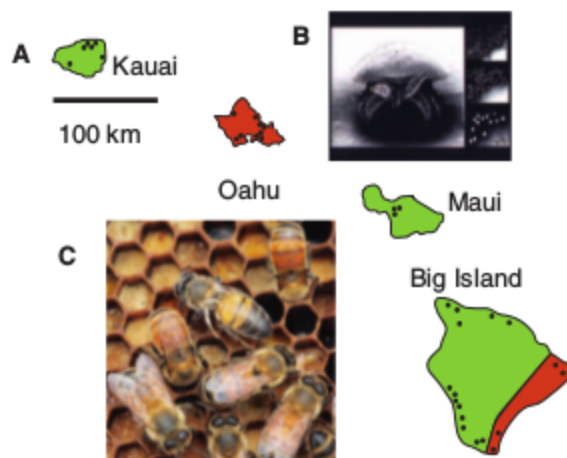


Fig. 1. (A) The four main Hawaiian Islands, showing the distribution of *Varroa* during 2009. Green and brown indicate *Varroa*-free and *Varroa* infested areas respectively. Dots indicate the location of each study apiary. By November 2010, *Varroa* was present throughout the Big Island. The co-occurrence of the *Varroa* mite (B) and DWV can result in overt symptoms of (C) deformed wings in honey bees, although many nondeformed bees also carry high DWV loads.

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Identifying the viruses causing mortality of honey bees in colonies infested with *Varroa destructor*

The number of honey bee (*Apis mellifera*) colonies in New Zealand has decreased markedly in recent years following the spread of *Varroa destructor* throughout the North Island. Similar losses have occurred in all countries where these parasitic mites have become established. However, overseas research has shown that colony losses are not due solely to the mites themselves⁽¹⁾, but also to honey bee viruses that the mites are able to acquire and transmit when they feed on the haemolymph of infected adult bees and developing brood⁽²⁾⁽³⁾⁽⁴⁾. This research suggests that the viruses are at least partially responsible for the mortality of the mite-infested colonies⁽⁵⁾⁽⁶⁾⁽⁷⁾.

Bees and insecticides

Subtle poison

Evidence is growing that commonly used pesticides, even when employed carefully, are bad for bees

Mar 31st 2012 | From the print edition



Review article

**Co-adaptation of *Apis cerana* Fabr.
and *Varroa jacobsoni* Oud.**

Werner Rath

Apidologie

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Scientific note

Scientific note: varroa mite eradication, the strange case of Gorgona Island

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varroa mite eradication / DWV titer / honeybee health / monitoring program / small island

ORIGINAL ARTICLE



Suppressed mite reproduction explained by the behaviour of adult bees

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SUMMARY

Suppressed mite reproduction (SMR) is a heritable trait of the honey bee (*Apis mellifera*) that can control the parasitic mite, *Varroa destructor*. The purpose of this study was to determine whether adult bees with the SMR trait affect mites in brood after cells are capped. Colonies with or without the SMR trait were each given a comb of newly-capped worker brood that was naturally infested with varroa. Each of 7 source colonies provided a comb of brood to at least one SMR ($n = 9$) and one control colony ($n = 8$). These combs were removed from their host colonies 8 days later and mite populations evaluated in cells with bee pupae that were >8 days post-capping. Colonies with SMR bees averaged 2.2% of their cells infested with mites; controls averaged 9.0%. Therefore, bees with the SMR trait apparently removed mites from capped cells. Of the mites that remained, the SMR colonies had a much lower rate of reproductive mites, 20% vs. 71%. This suggests that bees with the SMR trait removed reproductive mites more often than they removed non-reproductive mites. When comparing only the number of mites that produced no progeny, the groups were almost identical averaging 1.2 and 1.3 mites per 100 cells of brood. This suggests that the SMR bees did not remove mites from brood cells if the mites did not lay eggs. By targeting the reproductive mites, bees with the SMR trait give the illusion that nearly all of the mites are non-reproductive. Therefore, our selection for a low frequency of reproductive mites may have produced bees that remove reproductive mites from capped brood.

Keywords: *Apis mellifera*, *Varroa destructor*, SMR, hygienic behaviour, parasitic mites, honey bees, resistance