Buckfast VSH – Single Drone Project - 2014 Results

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Stichting Arista Bee Research

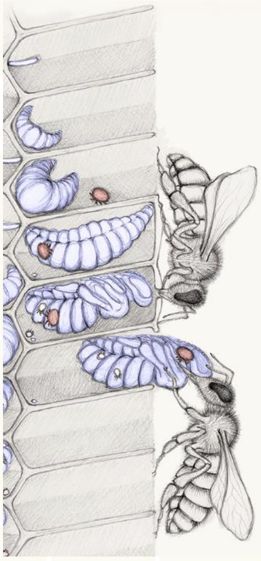
Boxmeer, February 11, 2015.

Introduction

The Western honey bee (*Apis mellifera*) is important for pollination of our food crops, but is being challenged by the highly damaging parasitic mite: *Varroa destructor*. The Western Honey Bee is not (yet) able to deal with this mite. The mite creates a hole in the exoskeleton, the armor of the bee and directly weakens the bee by sucking hemolymph ("insect blood"). Through the resulting hole, viruses and bacteria can enter the honeybee and cause disease. Untreated colonies often collapse within 2 years from the consequences of the rapidly growing Varroa population.

Several chemical treatments have been developed against the mite. The treatments of colonies are laborious, have variable results, can become ineffective over time (mites build up resistance) and do not only effect the mites but can also harm the bees. Despite treatments, the *Varroa destructor* is still considered as the largest contributor to (winter) colony losses.

There is a broad consensus that a longer term solution is needed to ensure a sustainable, healthy honeybee population. It has been shown that a natural solution is possible: untreated honey bees in tropical, isolated environments develop certain levels of Varroa resistance. A heavy selection pressure (no treatments by beekeepers, so a lot of Varroa) is combined with a favorable climate for the bees without a winter. This ensures that a certain percentage of colonies can survive, at least long enough to ensure the creation of offspring (swarms, new colonies). These colonies have likely inherited one or more traits that help them to keep the Varroa infestation under control.



Also research has yielded colonies with favorable traits that can keep the number of Varroa mites in the hive below damaging levels. The best results so far are achieved by the United States Department of Agriculture (USDA) in Baton Rouge, Louisiana (USA), by selecting (Western) honey bees with Varroa Sensitive Hygiene (VSH) behavior: these bees can detect the (reproducing) Varroa in the brood. As these VSH bees remove the infested brood, no Varroa offspring is produced. The effectiveness of this mechanism is also shown in a different bee species, the Asian honeybee, *Apis cerana*, which is fully Varroa resistant.

The USDA has been able to create a research population of colonies which has a high expression of the VSH behavior and has very low mite infestation levels while not being treated. This research population is used to further develop selection techniques as for example the development of genetic markers.

The genetic base of the established USDA-VSH colonies is very narrow as selection has only been limited to this trait and no resources have been available yet to structurally integrate this trait in a broader base of the honey bee population.

Arista Bee Research has been founded to further integrate the VSH trait in a broad representation of the honeybee population in cooperation with beekeepers, institutes, universities and other stakeholders.

In this report the first year results of the Buckfast VSH – Single Drone project are being presented.

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Material & Methods

Project Team creation

At the end of 2013 a project team was created, composed of Buckfast beekeepers from Belgium, Luxembourg, Germany, France and the Netherlands. The team has a large amount of experience in breeding and artificial insemination. Furthermore the members of this team were already performing selection of colonies based on lower counts of Varroa fall after treatment, were performing hygienic tests and had successfully imported semen from USDA-VSH colonies.

Learning the Techniques

To understand the background of VSH and the techniques for selection, the USDA in Baton Rouge was visited twice (the first time in 2013 by Ralph Büchler and BartJan Fernhout, the second time in 2014 by Renaud Lavend'Homme and BartJan Fernhout). Tom Rinderer, Bob Danka and colleagues shared their knowledge on Varroa Resistance, their VSH program, characteristics of the VSH colonies, Single Drone Insemination, characteristics of Single-Drone colonies and the Genetic Marker development. During the second visit, also John Harbo (retired from USDA), the scientist who started the (USDA-) VSH project, also shared his knowledge on the selection of VSH honeybees. Both the USDA as well as John Harbo have continued their support during the project.

Pre- selection

To increase the probability of finding bees with a greater than average level of the VSH trait, both the mother line as well as the father line of the test-colonies were pre-selected. The beekeepers were free to make their own pre-selection. In general, good production colonies with lower amounts of Varroa (amount of dead Varroa after treatment or survival after non-treatment) and in most cases with good hygienic behavior (Liquid Nitrogen test) were selected as mother or father line.

Background

Two different backgrounds of bees were used in this project.

As a positive control and possible future source of VSH, a two-times-consecutive-USDA-VSH inseminated line was used. After two rounds of insemination this line should be 75% USDA-VSH on average (with 25% Buckfast which was used as the base). From this USDA background 24 colonies were tested.

The background of the second group, the main target of this project, consisted of pedigree-based Buckfast lines. From this EU background, 93 colonies were tested.

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Creation of the Test Colonies

Test colonies were either created with bees already heavily infested with Varroa (untreated in the winter(s) before) or extra mites were added after establishing the colony. Most of the USDA-background bees received extra mites by transferring some of their young, open brood in heavily infested colonies for the period of 1 week before putting them back in the test colony. The EU-background colonies were either already heavily infested from the start or got extra mites by harvesting mites from infested colonies with powdered sugar. After sieving, around 100 mites were put on water drenched paper towel to remove the remaining powdered sugar from the mites. This paper towel with the mites was put in the test-colonies after which the bees started to feed on the sugar dissolved in the water in the paper. The mites quickly jump on the feeding bees, most of them within 10-15 minutes.

The colonies were kept in small hives (most of them “Mini-plus”, which is the equivalent of 1½ standard frame Dadant) to ensure queens would not run out of semen too quickly, as these queens received only about 10% of the amount of semen a multi-drone queen would.

Single Drone Insemination

The Single Drone Insemination (SDI) is *the* key to quickly increase the VSH trait (or any other trait for that matter) in a population of bees. As the queens are inseminated with only one drone - instead of normally around ten – one is ensured that all worker bees have the same mother and the same father. This will strongly reduce the variability of the genetic background of the worker bees as only from the queen’s side genes can come from two sets of chromosomes (female bees are diploid). From the drone’s side (which is haploid), only the same set of genes can be inherited by the worker bee. This will then result in the worker bees from a single colony (who are sisters) being on average 75% genetically identical, as all the workers will inherit the same genes from their father and only the genes inherited from their mother will differ and that difference will be 50% on average. This will create a very uniform colony with worker bees expressing a very similar behavior.

In a normal multi-drone colony with multiple different fathers, sisters can share as few as 25% of the same genes (should the fathers not be related). This mix of different worker bees can be beneficial for the (production) colony as it has multiple behaviors which can be usefull in different circumstances. However this makes the selection of a specific behavior much more complicated.

The SDI technique ensures that the VSH trait can be measured as there is not a large mix of workers with different backgrounds. A SDI-colony will more clearly show low, moderate or high VSH levels.

The SDI method facilitates further breeding as the daughter-queens of these test-colonies will have the same genetic background as the worker bees that were performing the desirable behavior of removing Varroa mites.

Once the VSH trait is established at the 100% level, normal multi-drone mating will be used to produce normal, large production size colonies.

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VSH brood counting

When the test colony consists of worker bees produced by the SDI - queen and Varroa infestation is high enough (this is around 2 months after establishing the colony), brood is checked for the size and the reproductive state of the mite population. If the bees have the VSH trait, they can detect and remove those mites that are reproducing (producing at least one daughter). However, even with the VSH trait present, the bees cannot detect mites that are not reproducing (so these remain in the brood).

The reproducing mites are detected by the bees within the first week after the brood is capped. In this stage the bee-brood is still a larvae or pupae with white or pink eyes. So only the older brood (purple-black eye, 7-12 days post capping) is used to investigate the presence and reproducing state of the mites.

In this project we measured the amount of reproducing mites (defined as foundress mites with at least 1 daughter) as well as the amount of non-reproducing mites (foundress mites which are dead, have only just eggs or a male) and determined the percentage of Non-Reproducing (% NR) mites. It has been shown by the USDA that the % NR measured is predictive for the level of VSH. For these measurements only infested cells with one foundress mite are used because it is very difficult to score infested cells which can contain both reproducing as well as non-reproducing foundress mites.

As a working hypothesis (developed by the USDA) it is assumed that the VSH behavior is at least regulated by two different genes (therefore a possible maximum of 4 alleles in each worker bee). More genes might be involved, but these might be present in most bees and for that reason less important to take into consideration. This working hypothesis is based on the observation that outcrossing of high VSH material with low or heterozygote VSH material will give a range of low, medium and high percentages of Non-Reproducing mites, correlating with high, medium and low levels of mites.

So a relatively high amount of non-reproducing mites is a strong indication for high levels of VSH (the bees have removed many of the reproducing mites, the non-reproducing are still in the brood). The colonies with very low mite levels and almost all of the mites found being non-reproducing, are considered to be 100% VSH. For 75% VSH the % NR is around 50%, for 50% VSH the non-reproducing mites is around 33%, whereas NR percentages of 25% and lower indicate the lower levels of 25 and 0% VSH.

% non reproducing mites	VSH alleles	VSH %
100	4	100
67	3,5	87,5
50	3	75
33	2	50
25	1	25
20	0	0

Table 1

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Results

USDA Background Queens

In total 24 test-colonies with a USDA-VSH background were tested for VSH. 23 colonies were created with the described one-drone method. For one colony multiple drones were used. 5 queens (4 single drone, 1 multi-drone) were classified as 100% VSH (see table 2). These colonies had only a very low number of mites (1-2%), which were all non-reproducing. No reproducing mites were found in purple-eye and black-eye pupae stage, despite the fact that these test colonies were infested twice with mites. This could be confirmed by establishing the infestation level of the younger pupae (the stadium where the mites are not removed yet). In this stadium the infestation was 5 to 10 times higher (5-20%).

In addition, 6 queens were identified with 75 and 87,5 % VSH. 2 queens were classified as 50% VSH, 2 with 25% and 9 queens had no or very low levels of VSH. The 0% VSH colonies had high levels of Varroa infestation, ranging between 8 and 53%.

VSH%	Queens		**USA Queens (background VSH USDA Research stock) positive control**	
100	5	}	11	High level of protection against Varroa
87,5	2			
75	4			
50	2		2	Moderate protection against Varroa
25	2	}	11	Low or no protection against Varroa
0	9			
	24			

Table 2

The results from the USDA Background material, now in the hands of European breeders, confirm and are in line with the results from the USDA in Baton Rouge. The potential of both the one-drone insemination and the VSH trait itself is clearly demonstrated by the very low mite infestation levels in high %VSH colonies.

European Queens

Though a first pre-selection was made, it was difficult to make a reliable prediction of the results as no extensive SDI - VSH trials have been performed in Europe. For that reason a relatively large group of queens (total of 93) was tested for VSH.

Based on the percentage non-reproducing mites, of the 93 of these queens, 11 showed high levels of VSH (10 of the 75% category, 1 of 87,5%, see table 3) with an additional 12 queens showing levels of 50% VSH. 69 queens showed low or no presence of VSH. So in total more than 20 queens have VSH levels high enough (>=50%) for the next phase in the breeding program.

VSH%	Queens	**European Queens (from high quality production stock) potential future breeding stock**		
100	0	}	11	High level of protection against Varroa
87,5	1			
75	10			
50	12		12	Moderate protection against Varroa
25	13	}	69	Low or no protection against Varroa
0	57			
	93			

Table 3

With the capacity at hand it was decided to select the best 12 queens of this group for further breeding. These best 75% and 50% VSH queens have been cool-truck-transported to Spain where a longer beekeeping season will allow us to create multi-drone, longer living, F1-offspring of these valuable queens. This is necessary as the SDI queens have a limited life expectation (having only 10% of the normal amount of semen available).

The drones of this F1-offspring will be used in 2015 to further increase the VSH levels in the Buckfast breeding stock - with the 100% level as our target.

The tested colonies had different Varroa infestation levels at the start as they were created by different beekeepers with different Varroa infestation levels in their starting material. Still, most of the 50 and 75% VSH colonies had lower mite infestations then the 25% and 0% VSH colonies from the same beekeeper. On average, the tested colonies (US+EU) in the 0% VSH group had a 33% infestation, the 25% group had a 27% infestation, the 50% group 22%, the 75% group 18% (11% without two out-layers- see below), and 1 % for the 100% VSH group. So also this data shows the relation between the level of VSH and the infestation level as at higher VSH levels, mites are being removed from the colony.

In the 75% group 2 colonies (created with very high levels of Varroa) still had high levels of Varroa infestation (64 and 73%) at the moment of testing. So either they did not have enough time to reduce the very high levels of Varroa or they might not belong to the 75% VSH group (being out-layers). Because of the high infestation levels we did not select them for further breeding.

The VSH classification and infestation results also confirm the USDA experience that the lower and moderate levels of VSH only partly reduce the mite infestation in colonies. Higher VSH levels are needed to have a significant reduction in mite levels, whereas with 100% VSH the mite levels become extremely low.

Conclusion

The 2014 results in this project show that the VSH behavior, shown to be present in USDA research stock, is also present in European high quality Buckfast breeding stock and can be brought to high percentages by using the Single Drone Insemination technique. The next step in the breeding program will be to further select towards 100% VSH in the EU breeding stock. Herewith we will also be able to confirm the current measured VSH levels in the selected queens. Once the 100% level is reached, normal sized, multi-drone production colonies will be created and thoroughly tested on other important traits (like honey-flow, gentleness, swarming tendency).

The presented results are furthermore very encouraging as a lot of high quality breeding stock and breeding experience is available in Europe. Breeding initiatives are supported by a large number of island and land mating stations. In addition, insemination techniques are already applied to support the pedigree-based breeding and can also be used in support of the VSH breeding.

In addition to the Buckfast stock used in this project, it is believed that enough of the VSH trait is also present in the Carnica breeding stock to reach high percentages of VSH when the SDI approach would be applied. Carnica initiatives (Beebreed, AGT) have already been selecting for more than 10 years for less Varroa fall and greater hygienic behavior using multi-drone approaches. The Kirchhain Bee institute performed a trial in 2014 with 60 colonies were both queens and drones came from four pre-selected queens (insemination by multiple drones from single queen). Also in this trial when measured higher than average non-reproducing mite percentages were found. Arista Bee Research and the Kirchhain Bee Institute will work together on further increasing the VSH levels in Carnica stock.

The experience in the US and now also in Europe shows that the described method can be successful, but that it is at the same time very laborious. The “silver bullet” for the breeding of VSH bees would be a set of genetic marker tests that could be used by the beekeepers and institutes to do the necessary selection. The USDA and other groups have started the development of these markers, but only a very limited amount of resources is currently available. Now that there is VSH material from both the US and the EU, it becomes even more feasible to develop these genetic markers. Genetic markers would not only strongly facilitate the breeding in the Buckfast, Carnica, Ligustica and Black bees, but would likely also facilitate the breeding of Varroa resistant stock in other (more local) lines of bees.

The selection for Varroa resistant stock has to be extended and repeated in as many as possible different lines of honeybees to ensure a rich biodiversity and to enable its broad application in the beekeeping community worldwide. Additional cooperation, resources and funds will be necessary to further convert the available knowledge in usable tools and a broad selection of Varroa resistant honey bees.

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Acknowledgements

The project team is very grateful for the ongoing theoretical and practical support from John Harbo and the members of the Arista Bee Research Scientific Committee, Kaspar Bienefeld, Ralph Büchler, Pim Brascamp, Bob Danka, Tom Rinderer and Bas Zwaan.

The project team would like to thank the “VSH-brood-counting Squad” for measuring the VSH level in the test colonies: Bas Fernhout, Ireen Fernhout-Roskam, Renaud Lavend’Homme, Gerbert Kos, Lisa Oude Grave, and the family of Renaud for taking care of the team during the counting in Belgium.

Furthermore the project team is very grateful for the creation of a Breeding station in Spain by Gerbert Kos, who is taking care of breeding a F1-generation of the high-VSH selected queens. With these queens we will be able to continue the project in 2015.