

2002 SARE Farmer/Grower Grant Report

Project Number FNE 02-408

[1.] Rearing Free Mated Mite-Tolerant Queens Without Chemotherapies

[2.] GOALS The principal goals of this project were to evolve a model of a honeybee queen rearing operation not dependent on chemotherapies and able to provide a source of much needed locally adapted Varroa mite and disease tolerant queens to beekeepers in the Northeast. The project sought to integrate one of the recent developments in identifying Varroa mite tolerant traits and availability of Varroa mite tolerant honeybee stock with advances in sustainable methods of managing Varroa mites and disease in honeybee colonies. We planned to rear queens and assay their Varroa mite tolerance in a sustainably managed apicultural operation.

It was an essential objective of this project to engage the cooperation of a group of hobbyist beekeepers in addressing some breeding control challenges presented by the biology of honeybees. Since honeybees mate in flight and choose their own time and place for mating, the queen breeder has relatively few ways to influence which drones breed with which queens. Queen breeders can use instrumental insemination (technically involved and a significant capital investment) and /or find ways to influence the number and quality of honeybee colonies in an area around where he keeps the virgin queens. This project was to rely on the cooperation of hobbyist beekeepers located within 1.5 miles of the queen mating yard to provide selected drones for the open mating of queens.

[3.] FARM / APIARY INFORMATION This project was based at the project leader's two beeyards which in 2002 included 19 full size colonies and 12 nucleus colonies (smaller hives for the housing and evaluation of virgin and new queens) distributed in two bee yards separated by 1.5 miles. The bee yards are located 800 – 1250 feet above sea level in a hilly wooded area several miles west of the Connecticut River Valley. The project leader has operated a multi product small professional beekeeping operation based on Patch Farm in Westminster West Vermont for more than a decade. He maintains the queen rearing activities at Patch Farm which is located centrally to the three hobbyist cooperators' bee yards. In this report the relatively isolated area including these five bee yards is called the "project neighborhood".

The agricultural land in this rural area is used primarily for sheep and horse pasture. The growing season is relatively short and bee forage limited to mostly wild flowers, clovers and flowering trees. Although the project leader has operated a beekeeping operation here for more than a decade the area is only marginally suited for honey production with a key advantage being relative isolation from commercial beekeeping traffic and its associated added bee disease and Varroa mite pressure. This area has a mix of rural residential and small varied agricultural uses which provides other advantages such as relatively minimal risk from agricultural pesticides or polluting industries.

For the 2003 beekeeping season the project leader had 15 full size and 8 nucleus colonies and the cooperators had 10 full sized colonies.

[4.] THE COOPERATORS This project brought together three hobbyist beekeepers with a small professional queen rearing operation all located within a 1.5 mile radius. One cooperator is a long time sideline beekeeper and retired dairy farmer with up to 4 colonies located in the project neighborhood. A second cooperator is a college biology teacher and

avid hobbyist beekeeper who keeps several colonies on a hillside in her back yard. The third cooperator is a full time professional horticulturist whose long practical experience with organic growing methods often informs his approach to managing several honeybee colonies at his Farm. He also provided assistance to the project leader, as "project collaborator", when performing hygienic assays.

In addition to the one-on-one training provided to the hobbyist-cooperators while requeening some of their colonies, each of the cooperators devoted more than 10 hours over the 2002 season to developing these requeening skills on their own with the remaining colonies slated for requeening in their yards.

The cooperation and enthusiasm of these three hobbyist beekeepers before and during this project proved essential in developing and implementing the concept of strengthening available breeding stock in an isolated area for the ultimate benefit of all colonies in that area.

[5.] WHAT WAS DONE This project involved three cooperators and a professional beekeeper with a total of 33 full sized colonies at 5 locations plus 12 nucleus colonies at the queen breeding yard. The project leader provided technical support to the three cooperators so their colonies could produce drones of selected stock to mate with the virgin queens reared during this project.

Queen Rearing The project leader in consultation with the technical advisor designed a breeding plan, selecting queens with desirable traits such as producing colonies which tend to build up strength early, mild temperment, disease and Varroa mite tolerance. Participants had begun requeening colonies for two years previous to this project using queens from a few different sources thought likely to carry Varroa mite-tolerance and other desirable traits. This requeening effort continued through the project period using both queens from outside sources and those reared and mated as part of the project activities. Just a few queens were chosen, from lines not closely related to the drone mother colonies, to provide eggs for rearing of queens (grafting). The queen mother colonies were managed so they could not produce drones that might inbreed with the daughter queens. Participants managed the other 30 colonies for drone production. Depending on the cooperator skill level or amount of labor available the drone mother colonies were either actively managed to produce quantities of drones through introduction of drone brood and drone foundation or simply allowed to produce drone brood without the drone brood culling that is typical practiced in honey producing operations.

The project resulted in 26 successfully open mated queens reared from 4 graftings. These daughter queens were evaluated in nucleus colonies to see if they produced viable brood then moved to some of the 30 full sized colonies to be evaluated several weeks later for hygienic behavior, temperment, honey production.

Hygienic Assay Honeybees naturally possess several behaviors and biological characteristics that help them to fight off or at least tolerate Varroa mite infestations. It is of course the purpose of this queen breeding program to identify and breed from colonies with these traits. Not all traits are thoroughly understood or easily identified. One trait, the hygienic behavior involves the ability of honeybee nurse bees to sense which brood cells are infested with Varroa mites and then remove the contents before the mites mature. (This particular trait is recessive and so the need for a queen rearing operation that provides plenty of drones with

the trait.) For this project we relied heavily on a technique for evaluating the degree of hygienic trait in queens developed by Spivak and Reuter called a “hygienic assay”. This technique uses liquid nitrogen to freeze-kill a small section of worker brood which the apiculturist then checks 48 hours later to determine the percentage of killed brood cells that the bees have cleaned out. If the bees removed a large majority of the cells they likely carry the hygienic trait.

The project leader consulted with one of the developers of the assay, Marla Spivak, during the final season of the project (March – July 2003) and learned of recent modifications to the published version of the assay which he subsequently adopted. Since August 2003, after the project funding period, he assayed all colonies checking on the number of larvae removed after 24 hours as well as after 48 hours with the objective of finding queens whose colonies remove 95% or more of the freeze killed larvae within 24 hours. Another improvement to the assay adopted as a result of this project is to assay the same queen more than once. The liquid nitrogen hygienic assay was and continues to provide the key measure of evaluating queens for Varroa mite tolerant traits in this queen breeding operation.

Another important trait associated with mite tolerance in honeybees is called Suppressed Mite Reproduction (SMR). It was recently observed by researchers, as learned during discussions with the project technical advisor, that colonies which tested well for the SMR trait were also hygienic. Given this intriguing but as yet unexplained link in the appearance of the two traits the project leader decided to forgo SMR testing and shift limited project resources to focus on well-executed hygienic assays.

Disease and Pest Management In order to reduce spread of disease and parasites via drifting (tendency of perhaps up to 15% of field bees to return to a hive in the yard other than the one departed from) between colonies within the bee yards the colonies were situated where possible at least several feet apart positioned at differing angles to the sun, with different heights and painted different colors. The colonies were kept on mesh bottom boards for Varroa mite reduction and monitoring. Participants monitored Varroa mite levels and employed powder sugar dusting as a Varroa mite management tool. In 2003 participants began using a newly approved essential oil based treatment, marketed as “Apilife VAR”, for management of Varroa mites.

Drone Congregation Areas (D.C.A.'s) It can be useful but not necessary to locate the places where queens and drones meet, airborne, to mate (D.C.A.'s). The purchase of a disposable helium tank greatly facilitated the search by providing easy access to a lighter than air gas to fill party balloons for the purpose of lifting caged queens into the air. Once airborne the queens will attract drones in search of virgin queens. A group of drones following the queen's scent can indicate the location of a D.C.A.. The project leader decided to eliminate the extra expense and time required to obtain queen pheromones (9-oxo-2-decenoic acid) as a drone attractant and used live queens held in cages suspended from the helium filled party balloons. The balloons were attached to fishing line and reel and in this manner the caged queen was maneuvered approximately 25 - 75 feet above ground across local topography and around trees. Despite these efforts the search for DCA's yielded no positive results in 2002 or 2003. The search for DCA's needs to cover a wide area: as time permits next season the project leader plans to continue searching using this technique.

[6.] RESULTS AND ACCOMPLISHMENTS

Requeening Even though the project plan/application anticipated that hobbyists would need help with the multi-step, technically demanding operation of requeening, this project suggests that it could take at least two years to requeen with hobbyist cooperators as they continue to gain experience with the procedure.

Evaluating Queens The project leader charted the results of the hygienic assays to showing percent of frozen-killed larvae removed after 48 hours in 2002. Of those colonies that were assayable (had enough brood cells close together at the time of the assaying) we had the following results at the end of the first season of the project: 45% of queens from a variety of outside sources in the project bee yards were hygienic. A similar percentage of project-reared queens were hygienic according to the assays in 2002.

If we had not had heavy losses (please see section below on “Varroa mite levels”) in the winter following these first assays we would have requeened non hygienic colonies in the fall and following spring while proceeding to rear more free mated queens. While it appears impractical at this stage to apply precise figures to the percentage of hygienic queens likely to result from open mating among a certain concentration of hygienic drone mother colonies we can safely assume that results will improve as more colonies are established with Varroa mite tolerant traits. In other words since the hygienic trait is recessive the hygienic virgin queens must mate with drones who also carry the trait in order for the mated queens colonies to hygienically control mites. Therefore it has been and remains an ultimate objective of project participants to establish mite tolerant stock in all colonies. Through the course of the 2003 season the project leader continued assaying and requeening colonies with the ultimate objective of replacing all queens with tested hygienic queens. As of the end of 2003 it looks like next season will offer the highest concentration of hygienic honeybee stock in the project neighborhood.

It seems good practice to evaluate all queens regardless of pedigree since not all queens sold as likely to carry the hygienic trait exhibited that trait when assayed during this project. Of equal importance in the search for breeding stock was the result of hygienic assays of package bee queens performed in 2003 which showed that 10% of the queens tested hygienic from a source making no claim of Varroa mite-tolerance.

With the help of detailed colony management notes it was possible to monitor the colonies for other traits such as temperament, apparent honey productivity, hive vigor as judged by tendency to build up colony strength and respond to nectar flows. Winter survival unfortunately was one trait not measured during this project period, but will likely be fully assessed in the spring of 2004.

Varroa Mite Levels / Colony Losses Allowing some Varroa mite pressure makes sense when testing the Varroa mite tolerance of honeybee stock. In several cases the literature suggests seeking out and breeding from colonies which survive heavy Varroa infestations. Several factors are worth taking into account as concerns quantifying and managing Varroa mite levels not least of all infestation pressure from outside an individual bee yard.

With the first autumn of this project it turned out that Varroa mite levels were discovered to be too high too late in the season to control using sugar dusting and we had very high colony losses in the winter of 2002-2003 compounded by prolonged very cold periods (please see section 7 “Growing Season”). Sugar dusting, as one component in a Varroa mite

management plan, requires (more so than do chemotherapies) early treatment before the annual, late summer boom in Varroa populations. Having anticipated this necessity of early monitoring and treatment the project nonetheless had insufficient person hours available during July and August to perform the labor intensive sugar dusting. A related factor was the use of an organic powdered sugar which because it did not contain cornstarch was prone to clumping. We re-pulverized the sugar using a modified mortar and pestle, but the project would have benefited from an appropriate commercial grinder or other more efficient tool for re-pulverizing the sugar powder.

The literature and the experience of the project participants show that successful Varroa mite management requires working with a knowledge of the annual cycle of Varroa populations which tend to build up over the summer in proportion to the brood rearing activity of the bees. However during the course of this project it became apparent that the cycling of Varroa mite populations over the course of two to three years can play a major role in Varroa mite management. Near the end of 2002 it was apparent that Varroa mite levels in the project neighborhood were medium to very high and rapidly booming. By making inquiries among hobbyist beekeepers (including some peripherally located hobbyists who we not participants in the project) within a several mile radius of the queen rearing operation the project leader learned that nearly all colonies were established in the area from (treated) package bees 2-3 three years earlier. In the preceding two seasons Varroa mite populations had been reestablishing, spreading between apiaries below or near probable treatment thresholds. Untreated Varroa mite infestation with associated viruses, known as Parasitic Mite Syndrome, reliably results in crashing and death of untreated susceptible colonies usually in the second or third year. In this case the third season required intensive management well beyond the degree of effort required from local beekeepers in the previous two seasons. The experience of this project suggests that in the presence of apiaries that are not annually and efficiently treated using standard miticides, and even in the presence of some Varroa mite tolerant stock in a relatively isolated locality, it behooves beekeepers to take a holistic approach to treatment choices. We need to observe and analyze what has been happening with Varroa mite populations over an entire locality over the previous few years not just the previous season.

As was shown by European researchers in the early and mid 1990's Varroa naturally travel very effectively between colonies separated by a few kilometers. This experience reminds us that from the Varroa mite's perspective honeybee colonies are more or less hospitable population centers accessible via thousands of airborne host bees engaged in drifting, robbing and probably other less understood behaviors that allow transfer of Varroa mites between honeybee colonies. Of course if Varroa mites can readily move to re-infest separate bee yards so too can they move between colonies in the same bee yard. If high Varroa mite levels are allowed in bee yards where colonies are of mixed degrees of hygienic behavior then even the most hygienic colony will experience constant infestation pressure which could it seems counteract the colony's own Varroa mite reduction efforts. It seems a fair speculation that for this kind of project the Varroa mite tolerance of a single colony would be compounded if virtually all the colonies in a bee yard were similarly Varroa mite tolerant thus reducing infestation pressure between colonies.

[7.] SITE CONDITIONS, GROWING SEASON This project coincided with a winter of unusually long periods of cold temperatures which combined with a local high point in the two or three year cycle of Varroa mite build-up (see section 6) resulted in very heavy winter

losses of colonies. Cooperators as well as other non-project hobbyists within a few miles of the queen breeding operation all sustained heavy losses consistent with levels of Varroa mite pressure built-up over 2-3 years since starting colonies from packages. The hills of Westminster West Vermont lack the temperature moderating effects of a river or lake valley which likely contributed to the length of time when cold temperatures confined bees within the hives restricting cleansing flights and adding to health stresses.

Another area of concern has to do with bee forage and developing agricultural practices on and near the farm where the queen breeding operation is based. For several years following 1989 when the project leader first brought colonies to the site, it appeared that wild and cultivated forage in the area could support more than 20 full size colonies, but that has changed especially in the two seasons encompassing this project. In the last few years the land use has changed to more intensive rotational grazing of sheep. Waste areas that previously provided rich bee forage with essential late season composite wild flowers have been renovated to add to the sheep pasturage on the farm. It now seems obvious that average annual bee forage in the area ranges between adequate and insufficient depending on the year. Forage problems most certainly affected project colonies and are a pressing concern for this beekeeping operation. It is hard to gauge precisely the impact forage problems had on the results of this project, but it is safe to assume that it was a contributing factor to colony strain in 2002. To address this problem the project leader is working with the sheep farmer to find methods to increase bee forage such as the increased use of white and sweet clovers in the pastures.

[8.] ECONOMIC CONSIDERATIONS The beekeeping literature describes that queen breeding uses hive resources, ultimately reducing the honey harvest from colonies involved in the queen rearing operation. This was borne out by our experience in 2002 – '03 when the project leader shifted more colony resources to queen breeding for this project. Add to this that bee forage is marginal as mentioned above and it is clear that the business plan for queen production in this type of setting cannot depend on annual significant honey crops. It is also apparent with this specific operation that, regardless of whether it is principally honey or queen producing, it requires an outlay of additional resources to work with landowners to increase bee floral forage.

[9.] [10.] NEW IDEAS AND THE NEXT STEP IN CONTINUED FREE MATING OF QUEENS WITHOUT CHEMOTHERAPIES Notwithstanding future success at achieving higher levels of selected stock in the neighborhood of the queen breeding yard it seems likely that additional control of mating would eventually become necessary for some purposes. For example it would be difficult to assess the results of cross breeding specific lines in an open mated operation consisting of a wide variety of honeybee stock. Instrumental Insemination (I.I.) provides an added degree of control over breeding. The project leader plans to use a combination of I.I. and free mating of queens with reduced dependence on cooperators ability to requeen. In time local cooperators will improve their stock, in part with I.I. queens from this operation and from others sources, thus improving the chances that recessive and additive mite tolerant traits will be propagated in the colonies of all local beekeepers within range of common DCA's (mating locations). At the end of the 2003 season the project leader began learning I.I. and setting up a small facility at the queen breeding yard in order to implement I.I. in the 2004 season.

[11.] OUTREACH The project leader has agreed to make a presentation to the beekeepers group which meets in Windham County at their next meeting during the winter of '03 – '04. This group includes about 40 small professional and hobbyist beekeepers for whom some of the project ideas and results could be applicable.

He plans to offer a report of this project to the VT Department of Agriculture's "Agriview" which reaches the members of the Vermont State Beekeepers Association. On 12 July 2003 the Brattleboro Reformer newspaper published a full page color "Photo Journal" which described the queen rearing activities of this project and gave credit to the support of SARE. The project leader submitted a summary of the activities and observations associated with this project to Vermont Vegetable and Berry Growers News for the October 2003 on line "final reports from the field".

[12.] DATE AND NAME OF PROJECT LEADER, author of this report:

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28 November 2003

FNE 02- 408		HYGIENIC ASSAYS 2002			J.T. Cunningham						
	Cleanout					Cells	Cells				
	Period				Date Re-	Empty	Empty	Total		Probably	
Assay Date	in Hours	Colony ID	Queen ID	Queen Info.	queened	Before	After	Cells	% Removed	Hygienic	
Patch Farm											
26-Sep-02	48.25	JC(10) PF	GI.254.11	SMR x Carn. '02	08-Jul-02	30	90	116	69.7674%	no	
3-Oct-02	72	T(20) PF.	Q80.01.28	< GI.MN.Hyg.28	14-Aug-02	40	76	116	47.3684%	no	
Littm. Yard											
8-Oct-02	23.5	BB(12) Litt	Q8.01.90	< Q8 Ital.Hyg	previous yr.	90	130	130	100.0000%	yes	
8-Oct-02	23.5	BB(20) Litt	BB.Lt20.02	<Willb.	25-May-02	46	120	120	100.0000%	yes	
Akaogi Yard											
10-Oct-02	48	East	JC2.02.82	Moth:SMRxRus	13-Jul-03	45	14		68.8889%	no	
10-Oct-02	48	Center	JC4.02.82	Moth:SMRxRus	14-Jul-03	73	97	153	30.0000%	no	
McCulough											
21-10-02	48.25	#1	BB.Mc1.01	Willbanks Package	25-May-02	90	153	153	100.0000%	yes	
21-10-02	23.16	#3	JC21.01.39	<GI.Russ.Hyg.Breeder	09-Nov-03	39	104	165	51.5873%	no	
Littlefield											
23-10-02	48.41	#1	JQ1.02.99	Moth:GI.It.Hyg.b-line	05-Jul-03	61	147	147	100.0000%	yes	
23-10-02	48	#2	JQ3.02.80	clipped	05-Jul-03	no brood			-		
23-10-02	48	#5	GL254.12	SMRXCarn, 5July	05-Jul-03	30	51	158	16.4063%	no	
23-10-02	48	#3	12.02.80	Lg. Blind	31-Jul-03	72	158	161	96.6292%	yes	

Apiary Data of Jeff T. Cunningham			FNE 02 - 408								
HYGIENIC ASSAYS											
P.F. Yard 2003											
Assay Date	Colony ID	Queen ID	Queen Info.	Date Re-queened	Cells Open Before	Cells Empty After	Total Cells	Cleanout Period In Hours	% Removed	Hygienic?	
2003 JC											
19-06-03	PF(6)	Wi	x		10	34	140	48	18.4615%		
19-06-03	PF(7)	Wi	x		24	97	157	48	54.8872%		
19-06-03	PF(2)	Wi	x		28	110	138	48	74.5455%		
19-06-03	PF(3)	Wi(PF3).03.	*		19	125	126	48	99.0654%	*	
AUGUST											
16 Aug. 03	PF(3)	Wi(PF3).03.	Best of Willbanks	21-May-03	20	128	157	24	78.8321%	x	1seal., 28 part. Remov.
			*		20	157	157	48	100.0000%	*	
16 Aug. 03	PF(2)	MSb10.03	Marla	21-Jul-03	34	105	147	24	62.8319%	x	34 sealed, 8 partial
			*		34	143	147	48	96.4602%	*	1seal.livel, 3 partial
26 Aug. 03	PF(1)	Str(PF1).03	Strachen N.W.C.	14-Jun-03	4	93	126	24	72.9508%	x	3seal., 1partial
			*		4	122	126	48	96.7213%	*	
27 Aug. 03	PF(7)	BW(PF7).03	B.Weav. Russ.Hyg.	26-Jun-03	8	132	136	24	96.8750%	**	1seal., 3 partial
			**		8	136	136	48	100.0000%	*	
28 Aug. 03	PF(6)	GL344(PF6).03	Glenn MINN.HYG.	19-Jun-03	15	127	136	24	92.5620%	**	1seal., 8 partial
			**		15	136	136	48	100.0000%	*	
29 Aug. 03	PF(5)	BW(PF5).03	B.Weav HYG.	23-Jun-03	32	100	146	24	59.6491%	x	16seal., 40 part.
			x		32	132	146	48	87.7193%	x	
29 Aug. 03	PF(4)	JC10(PF4).03.325	< GL.325 crip. SMR Rus.	> 19-Jun-03	27	133	155	24	82.8125%	x	2seal., 20 part.
			*		27	155	155	48	100.0000%	*	

From Jeff T. Cunningham, Honeyhunter Apiaries (small professional producer of queens, apicultural produce and sometimes apicultural researcher using organic methods), Westminster West, VT

This season's honey and pollen harvests from my bee yards and those of several hobbieists in Westminster West area appear lower even than recent dry year's harvests probably due to the rain's limiting effect on foraging flights. Cyclically heavy mite loads here last year contributed to heavy winter losses. We replaced most dead colonies with package bees in the spring which meant that mite loads were exceptionally low this season and we will not need to treat for mites until next year.

How to treat for Varroa mites remains the principal challenge to sustainable beekeeping. Conventional treatments with a synthetic pyrethroid are now losing effectiveness so the EPA recently allowed the rather extreme choice of an organophosphate product. On the other hand Apilife Var, a European essential oil product, was approved for use in VT this year. My operation makes use of several biotechnical methods and sustainable practices to manage disease and mites (e.g. powdered sugar dusting, mesh bottom boards, heat therapy).

BREEDING FREE MATED MITE TOLERANT QUEENS WITHOUT CHEMOTHERAPIES:

Since the spread of Varroa mites, honeybees no longer survive more than three years in this climate without human management. The most necessary condition for long term survival of honeybees is the development of mite tolerant stock through selective breeding of queens. Westminster West does not have the climatic advantages for honeybees of valley regions, but one advantage we have critical to queen breeding is our isolation from commercial scale beekeeping operations. Without commercial beekeeping in this area and through cooperation with and among hobbyist beekeepers we have a potentially high degree of influence over the breeding of free mated queens.

This an exciting time in the development of mite tolerant traits in honeybees; experimental lines of mite tolerant stock are readily available. With the help of a grant from the USDA's Sustainable Agriculture Research and Education Program and the essential cooperation of hobbyist beekeepers I have been working the last few years to identify and increase the number of queens who produce mite tolerant colonies in this neighborhood. Our goal is that queens and drones on mating flights will accumulate the traits which produce colonies that can survive without chemotherapies. Eventually feral colonies, which in VT died out from mites in the 1990's, will reestablish from escaped swarms of mite tolerant stock. We expect horticulturists will eventually benefit from this burgeoning of the available pollinators.

ABOUT FLORAL FORAGE FOR HONEYBEES AND OTHER BENEFITIALS

I am increasingly concerned about loss of floral forage in this hilly, wooded area where beekeeping has long been only marginally productive at best. Changing land use, successional reforestation, increasingly efficient intensive grazing are reducing forage for many beneficial insects in some areas of VT. I believe we see the stress first in honeybees, an indicator species, already under pressure of mites and disease, in hilly woodland regions with relatively little floral forage areas and moderate to light horticultural use like Westminster West. It would certainly help native bees, honeybees, beneficial wasps and beetles if more "waste areas" and flowering meadows were allowed or encouraged to bloom through the season until heavy frosts. Dandelions are especially critical to early build up of honeybee colonies. Flowering trees such as basswood, cherry, locust, catalpa also are important sources of nectar. And of course flowering cover crops, especially white and sweet clovers, can provide much needed nectar and pollen mid to late season.