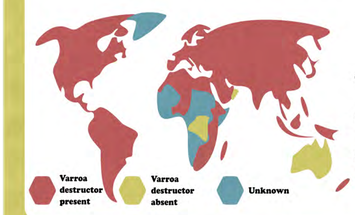
BeeT

Helping Honey Bees Fight the Mite *Varroa destructor*

Honey bees are responsible for the diversity of our food. However, there has been a **sustained loss of bee colonies** in the western world for at least 10 years[1]. Talking to beekeepers and experts, we found that the major cause is ***Varroa destructor***: a parasitic mite that weakens the bees and spreads diseases.



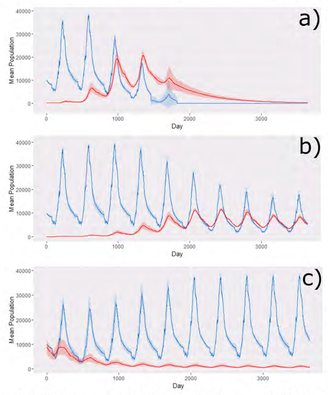
To save the bees, we need to **combat *Varroa*!**

Current treatments against *Varroa* have limited effect, require careful dosage, and may contaminate honey[2]. Moreover, beekeepers do not have the resources, time, or experience to use them properly. To target *Varroa* more effectively and secure our food supply, we designed BeeT.

BeeT is a **specific**, **regulated**, and **safe** *E. coli*, engineered to kill *Varroa* inside the bee hive.

Application

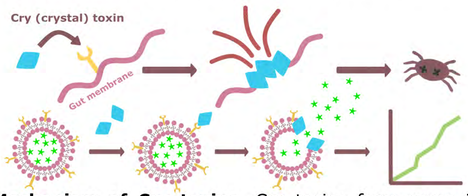
During the development stage of BeeT, we were unable to do real-world testing. Hence, we used a model to simulate its application. **Beehave** is an existing model that simulates bee colonies[4], including the effects of *Varroa destructor*. We adapted it by adding the effects of BeeT on *Varroa* and *Varroa*-transmitted virus dynamics. The model simulations tell us that when we apply BeeT, even when its toxin is suboptimal, the bee colonies will survive.



Population dynamics of bees without BeeT (a), barely surviving *Varroa* infestation (b), surviving a high starting population of *Varroa* mites. Blue lines indicate worker bees, Red lines indicate *Varroa* mites.

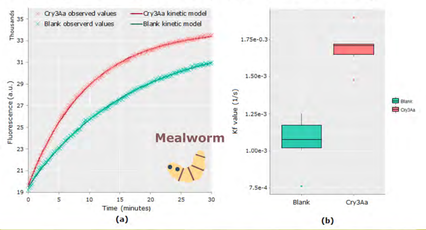
Specificity

BeeT needs to kill *Varroa*, but not affect anything else. We chose Cry toxins because they are very species-specific. They kill insects by forming pores in their midgut, but no variants targeting *Varroa* are known. Since we cannot work with *Varroa* mites in the lab, we developed an assay to screen for new toxin variants.

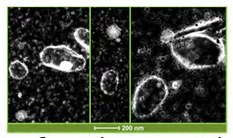


Mechanism of Cry toxins. Cry toxins form pores in the midgut of insects by binding to specific receptors. The *in vitro* assay uses fluorophore-filled vesicles that were prepared from the insect midgut. Upon addition of an effective toxin, the fluorophores are released, which can be measured.

To test if our method is valid, we used mealworm vesicles and Cry3Aa that is specific for this insect. We observed that the speed of fluorophore release (K value) was higher when Cry3Aa was added, confirming the validity of our assay.



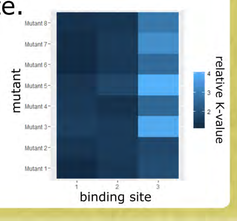
Since no *Varroa* specific Cry toxins are known[3], we made a random mutant library of Cry3Aa, targeting the three binding sites of the protein. We assessed their toxicity for *Varroa* using our *in vitro* assay with mite derived vesicles.



TEM image of Varroa midgut vesicles. Images were made by team TU Delft 2016.

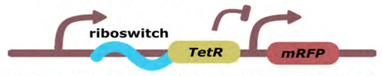
We found several mutants indicating increased toxicity towards *Varroa* mites, as can be seen in the heatmap. The results implied that binding site three has a high impact in Varroa toxicity, as the K value varies the most for this site.

Cry protein domains and binding sites (1,2 and, 3)

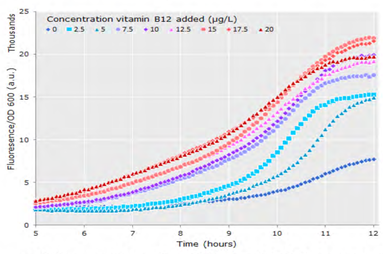


Regulation

BeeT should only produce the toxin when *Varroa* is present in the hive. Therefore, we designed a B12 riboswitch system. *Varroa* feeds on bee hemolymph that contains B12, hence we expect higher B12 levels when *Varroa* is present. We coupled the system to mRFP production and observed that higher B12 levels lead to increased fluorescence.

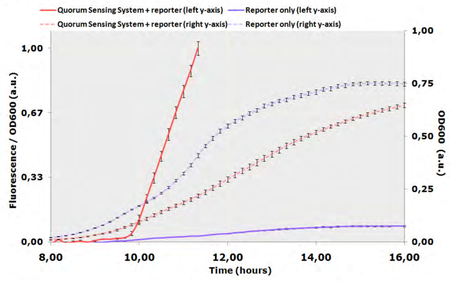


B12 riboswitch coupled to *mRFP* expression



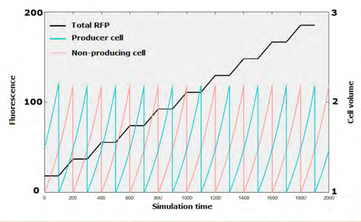
Response of the system to different B12 concentrations

Overexpression of Cry toxins can be toxic for *E. coli*. We separate the growth phase of BeeT from the toxin production phase with quorum sensing. We show that gene expression can be regulated by cell density using a GFP-reporter.



Additionally, we designed the quorum sensing system to maintain subpopulations of toxin producing and non-producing cells. We expect that producing cells die due to high toxin concentrations, but that they can be replaced by non-producing cells. This way, BeeT can finish its job. We used a computational model to describe this behaviour.

Computational model of the subpopulation. RFP represents the toxin. We follow one cell for each sub-population.



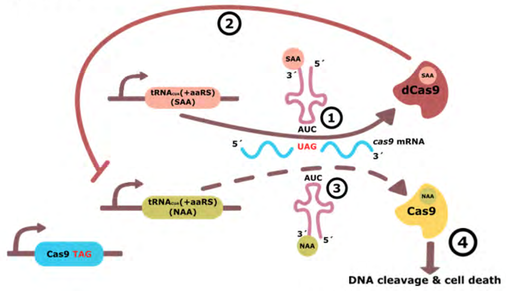
Safety

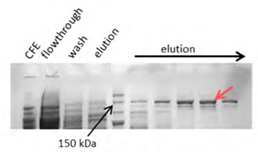
To improve the safety, we designed BeeT to contain it to the hive.

The riboswitch alone would not be able to produce an on/off response which effectively kills the mite. To establish a stable system, we created a toggle switch. This switch ensures that there is strong toxin production as soon as the mites are detected. However, if BeeT escapes from the hive, the light shuts down toxin production. Additionally, a light kill switch is activated.

Fluorescence over OD of mRFP controlled by our Fixk2-hybrid promoter. Measurements were taken after culturing for 24h in darkness. Error bars show the standard error among three technical replicates.

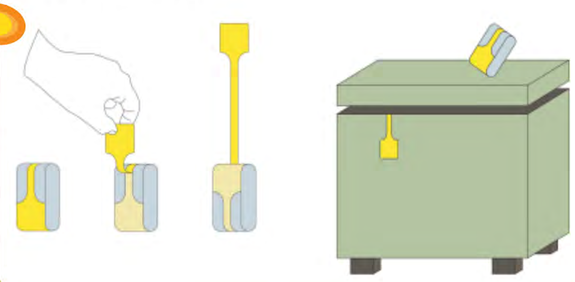
To prevent horizontal gene transfer, we used Cas9 to cleave heterologous DNA when BeeT leaves the hive. We constructed a system that relies on a synthetic amino acid (BipA) that is supplied to the hive. In the presence of BipA, dead Cas9 is formed which prevents production of active Cas9. Once BeeT escapes, active Cas9 is produced.





Above, the Cas9 kill switch mechanism. Left, a gel showing dead Cas9-BipA purified by His-tag chromatography.

We collaborated with the Design Academy Eindhoven, during which we educated each other about our disciplines. One student, Thieu Custers, designed a visual prototype of the **BeeT bag**:



Events we attended:

We attended and organized a variety of events where we talked and discussed about synthetic biology´s future and safety. As a result, we made a university magazine edition where we have written light-hearted stories based on synthetic biology in 2030.

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References

1) Seitz, N., Traynor, K. S., Steinhauer, N., Rennich, K., Wilson, M. E., Ellis, J. D., ... & Delaplane, K. S. (2016). A national survey of managed honey bee 2014–2015 annual colony losses in the USA. Journal of Apicultural Research, 1-12.
2) Serra Bonvehí, J., Ventura Coll, F., & Ruiz Martínez, J. A. (2016). Residues of essential oils in honey after treatments to control Varroa destructor. Journal of Essential Oil Research, 28(1), 22-28.
3) Alquisira-Ramírez, E. V., Paredes-Gonzalez, J. R., Hernández-Velázquez, V. M., Ramírez-Trujillo, J. A., & Peña-Chora, G. (2014). In vitro susceptibility of Varroa destructor and Apis mellifera to native strains of Bacillus thuringiensis. Apidologie, 45(6), 707–718.
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